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A genome-wide association study identified candidate genes associated with egg quality traits in Muscovy duck

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Abstract

Background Egg quality directly determines embryo development in meat-type poultry. However, it is difficult to directly select the egg quality of Muscovy duck. The genes and SNPs associated with egg quality screened by GWAS can be used for molecular breeding and accelerate the progress of selection in Muscovy duck.

Result 295 Muscovy ducks were used for whole genome sequencing, and a total of 6,131,623 SNPs were obtained for further analysis. The heritability of egg quality ranged from 0.01 to 0.41, in which egg weight (EW) was 0.19, albumen weight (AW) was 0.16 and the yolk weight (YW) was 0.27. The genetic correlation of EW and AW, EW and YW, and eggshell thickness (EST) and eggshell strength (ESS) were 0.65, 0.51, and 0.74, respectively. Phenotypic correlations between egg quality ranged from −0.13 to 0.17. A total of 68 SNPs significantly associated with EW were located within the genes *PSMG4*, *SLC22A23*, *DNAH5*, *FABP6*, *ADAMST17*, *IGF1R*, *NTRK3*, and *SCAI*. The linkage disequilibrium (LD) analysis identified 2_75684453_C > G, 2_76305509_A > G, 2_76350118_T > A, 11_3834664_C > T, 11_4339778_C > T, and 11_8079686_C > T as tagSNPs to represent the significant SNPs. Fifty SNPs significantly associated with YW were located within the genes *XKR6*, *DNAJC24*, *SNCB*, *UNC5A*, *MAD1L1*, *NOTCH1*, and *WDR7*. The SNPs 14_9186714_C > T, 14_9199818_A > G, 15_5452098_C > T, and 18_9038052_C > T were selected as tagSNPs. Fifty-four SNPs significantly associated with albumen height were located within the genes *LIN9* and *NID1*. The SNP 3_17718980_A > G was selected as the tagSNP. The significant SNPs associated with eggshell strength were located within the genes *CLPX*, *EPHA5*, *ZBTB44*, *NOL6*, and *UBAP1*. The SNPs 25_1996726_A > C and 25_2078328_A > G were selected as tagSNPs. Genes associated with egg quality were significantly enriched in the positive regulation of the BMP signaling pathway in the GO enrichment analysis of biological processes. The KEGG enrichment analysis suggested that the SNPs located genes were significantly enriched in Axon guidance, Endocrine resistance, and Progesterone-mediated oocyte maturation.

Conclusion Some tagSNPs were identified that may be useful for molecular breeding of egg quality. *RNF423*, *RNF220*, *IGF1R*, *SLC22A23*, *WDR7*, and *NTRK3* may be candidate genes for egg quality traits in Muscovy duck.

Keywords Muscovy duck, Egg quality, GWAS, Molecular breeding

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Introduction

The Muscovy duck (Cairina moschata) is indigenous to the tropical areas of Central and South America. Its body weight is larger than meat-type ducks and similar to geese [1]. As for its large size, high meat yield, and low-fat content, Muscovy duck has become an outstanding meat duck breed [2]. Muscovy duck is highly adaptable to the environment, can be reared independently of aquatic systems, and shows greater resistance to high temperature than Pekin duck [3]. Muscovy duck eggs have thicker, denser shells, more durable membranes, and larger yolk [4].

Egg quality represents many characteristics of eggs, which affect storage time and hatchability [5, 6]. The albumen and yolk can provide nutrients for embryonic development [7]. The proper mechanical strength and thickness of the eggshell prevent water loss and microorganism invasion of the egg in the collection, classification, and transportation process, protect materials in the egg, and promote development of the embryo [8]. Even, studies in broilers have shown that albumen content affects body weight and carcass weight after hatching [9]. Therefore, proper egg quality is highly important for incubation in Muscovy duck.

Egg quality can be selected by its weight, shell color, shape, etc. However, the yolk and albumen could hardly be selected with no damage. Currently, there have been some molecular markers have been found for egg quality selection. *Leucine-rich repeat-containing 75 A* (*LRRC75A*), *core histone macro-H2A.1* (LOC101795967) and *neurogenin 1* (*NEUROG1*) were associated with the Eggshell index (ESI), LOC106014427 and *transcription factor 4* (*TCF4*) were associated with the Eggshell thickness (EST), *potassium voltage-gated channel subfamily H member 8* (*KCNH8*), *insulin receptor substrate 1* (*IRS1*), and LOC106018641 were associated with yolk color (YC), *synapse differentiation-inducing 1-like* (*SYNDIG1L*), *HYDIN axonemal central pair apparatus protein* (*HYDIN*), *collagen type I alpha 2 chain* (*COL1A2*), *FTO alpha-ketoglutarate-dependent dioxygenase* (*FTO*), and *forkhead box L1* (*FOXL1*) were associated with egg weight (EW), *collagen type VI alpha 3 chain* (*COL6A3*), *lysine-specific demethylase 7 A* (*KDM7A*), LOC101802169, and *sperm-associated antigen 16* (*SPAG16*) were associated with yolk weight (YW), and *mucin 6* (*MUC6*) and *low-density lipoprotein receptor class A domain-containing 3* (*LDLRAD3*) were associated with the albumen composition [10–13].

Molecular breeding requires enough molecular markers, and Genome-Wide Association Study (GWAS) can effectively search for SNPs associated with traits [14]. The egg quality is controlled by polygenes with different genetic markers in different varieties and populations. Therefore, the exploration of more populations and more genetic markers can provide more references for molecular breeding. As well as the quality control of egg quality can also help to improve the hatchability.

Materials and methods

Animal samples

The experimental Muscovy ducks were provided by Anqing Yongqiang Muscovy Duck Co., Ltd., Anhui, China. The ducks were raised in small pens, with 1 male and 5 females per pen. All the ducks were provided free access to food and water and were provided 16–18 h of light during the laying stage. The nutritional levels of the ducks were met by the National Research Council (NRC). The numbered cages were placed in each pen. Ducks with eggs were placed in the corresponding cage from 8:00 pm to 8:00 am, and the next day, the eggs were identified by touching the lower abdomen. At 51 weeks of age, three eggs were collected from each of 295 ducks for egg quality measurement. Blood from the wing vein was collected using a 2 ml anticoagulant disposable vacuum blood collection tube containing EDTA-K_2 and a blood collection needle with a diameter of 0.55 mm. Blood was used for DNA extraction and genome sequencing. All animal experimental procedures in this study were approved by the Animal Welfare Committee of Anhui Agricultural University with the assurance number SYDW-P20230823021.

Egg quality measurement

Egg weight (EW), albumen weight (EAW), and yolk weight (YW) were measured via an electronic scale with an accuracy of 0.01 g. The long axis diameter (LAD) of the egg, the short axis diameter (SAD), and eggshell thickness (EST) were measured via a vernier caliper (150 mm, Sanliang, Guangdong, China). The egg shape index (ESI) was calculated as the ratio of LAD to SAD. The eggshell thickness was averaged by measuring the long, medium, and short axes. The yolk color was measured via a Roche Yolk Color Fan (Bulader, Beijing, China). The albumen height (AH) was averaged by measuring the middle of the yolk edge and the thick albumen edge, respectively, via a vernier caliper (150 mm, Sanliang, Guangdong, China). All fresh eggs were measured within 24 h.

DNA extraction and whole-genome resequencing

The DNA extraction and sequencing are carried out by Novogene Co., Ltd. DNA was extracted via the phenol–chloroform method. The quantity and quality of the DNA were detected via a NanoDrop 2000 (Thermo Fisher, USA) and agarose gel electrophoresis. Illumina NovaSeq 6000 PE150 was used for sequencing, with an average sequencing depth of 10X (Novogene, Beijing, China). The raw reads were filtered via NGSQCTool v2.3 with default parameters [15].

Variant discovery and genotyping

Clean reads were mapped to the reference genome of the Muscovy duck (ASM1810499v1) in the NCBI database via BWA v0.7.17 [16]. The obtained BAM files were sorted using Samtools, and then PCR duplicate sequences in these files were removed with the same tool [17]. SNP calling was performed via GATK and then filtered via VCFtools (minor allele frequency (MAF) > 0.01, genotype missing rate < 0.1) [18, 19]. The "HaplotypeCaller" was used to detect variants and generate GVCF files for each sample. The "CombineGVCFs" was then employed to merge the GVCF files of all samples, and the "GenotypeGVCFs" tool was used for joint genotyping, resulting in an original VCF file containing SNP variant information. Subsequently, the "SelectVariants" was utilized to extract SNP variant sites from the original VCF file, and the "Variant Filtration" tool was used to perform filtering on the SNPs with the set parameters "QUAL < 30, QD < 2.0, MQ < 40.0, FS > 60.0, SOR > 3.0, MQRankSum<-12.5, ReadPosRankSum<-8.0". The SNPs with missing genotypes were imputed via Beagle v5.0 [20].

Genetic parameter estimation and

Genetic correlation and heritability were calculated using the GCTA (v1.94.1) [21]. First, the Genetic Relationship Matrix (GRM) was calculated. Then, the restricted maximum likelihood (REML) method was used to estimate the genetic variance and environmental variance for the calculation of heritability. The phenotypic correlation was calculated using the formula.

$$\mathrm{r_p} = \mathrm{r_g}\sqrt{\mathrm{h1}^{*}h2}$$

where "r_p" represents the phenotypic correlation, "r_g" represents the genetic correlation, and "h2 and h2" stand for the heritabilities of the two traits, respectively. GCTA (1.94.1) was also used for PCA of population structure and heritability estimation [21]. The visualization of the first two principal components in the form of a scatter plot was created using the "ggplot2" package in R [22].

Genome-wide association analysis and annotation of significant SNPs

The GWAS was performed by using the following linear mixed model (MLM) in GEMMA [23].

$$\mathrm{Y} = \mathrm{W}\alpha + \mathrm{x}\beta + \mu + \varepsilon,\ \mathrm{u} \sim \mathrm{MVN_n}\left(0,\ \lambda\tau^{-1}\mathrm{K}\right),$$
$$\varepsilon \sim \mathrm{MVN_n}\left(0,\ \tau^{-1}\mathrm{In}\right),$$

where y is an n-vector of quantitative traits for n individuals; W =(w1,....., wc) is a n×c matrix of covariates (fixed effects) including a column of 1 s; α is a c-vector of the corresponding coefficients including the intercept; x is an n-vector of marker genotypes; β is the effect size of the marker and is an estimate of the marker/SNP additive effect; u is an n-vector of random effects; Ɛ is an n-vector of errors; τ^{-1} is the variance of the residual errors; λ is the ratio between the two variance components; K is a known n × n relatedness matrix; and In is an n × n identity matrix. $\mathrm{MVN_n}$ denotes the n-dimensional multivariate normal distribution.

Manhattan plots and Q–Q plots were used for the visualization of the GWAS results, which were visualized via the "cmplot" package of R [24]. The suggestive threshold values of the Manhattan chart are 3.6e-6 (1/N) and 1.8e-7 (0.05/N), respectively. The "N" was the total number of SNPs after the LD filter was performed via PLINK v1.90 (N = 276457, --indep-pairwise 50 10 0.2) [25]. Owing to the reference genome, ASM1810499v1 has no annotation file. The sequences of 5,000 bp from both the upstream and downstream regions of significant SNPs were blasted in Ensembl (https://www.ensembl.org/) against the refer ence genome CaiMos1.0 (GCA_009194515.1) to identify genes located nearby. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the annotated genes was conducted on the "Wei Sheng Xin" online platform (https://www.bioinformatics.com.cn/) [26].

Runs of homozygosity analysis

The Runs of Homozygosity (ROH) analysis of the population was conducted using the PLINK v1.90 with the following specific parameters: "plink --bfile test --homozyg --homozyg - density 50 --homozyg - gap 100 --homozyg - kb 500 --homozyg - snp 50 --homozyg - window - het 1 --homozyg - window - snp 50 --homozyg - window - threshold 0.05 --chr - set 29 --allow - extra - chr --chr 1–29 --out test". Here, "test" represents the population variation information file in PLINK format. The distribution plots and statistics of the number and average length of ROHs were generated using Excel. The chromosomal distribution plots of ROHs were created using the "ggplot2" package in R [22]. ROH segments present in 80% of the individuals were defined as hotspot ROHs and data processing was carried out using the R package "data. table" [27].

Linkage disequilibrium and statistical analysis

Linkage disequilibrium (LD) decay analysis was performed by using PopLDdecay-3.42 [28]. Significant SNP haplotype analysis, visualization, and tagSNP identification via the software packages hapviewer and LD block [29]. The LD is represented by D's statistic. The solid spine of LD (RR/D') is used to determine the LD block. Correlation analysis and visualization were performed via the "corrmorant" package of R [30]. Visualization of the density of SNP distribution on chromosomes via the

"CMplot" package of R [24]. Egg quality traits are displayed as the means and standard deviations. Differences between the phenotypes of different genotypes of the designated SNPs were analyzed via ANOVA (Prism 8.0, GraphPad Software, Boston, MA).

Result

Egg quality traits and their correlation analysis

The coefficient of variation (CV) of AW, AH, EST, ESS, and YC was greater than 10%. The heritability of Muscovy duck egg quality belongs to the medium and low heritability levels, ranging from 0.41 to 0.01 in Muscovy duck (Table 1, Table S1). There were significant positive correlations among EW, LAD, SAD, AW, YW, and AH, all of which are traits related to egg size (Fig. 1). EST was positively correlated with ESS and YC ($P < 0.05$). The data of all these traits met a normal distribution and can be used for GWASs.

Genetic and phenotypic correlation estimation of egg quality

The genetic and phenotypic correlations between EW and AW were 0.64 ± 0.30 and 0.11. These were 0.51 ± 0.22 and 0.12 for EW and YW, 0.95 ± 0.31 and 0.15 for SAD and AW, -0.54 ± 0.27 and − 0.03 for YW and YC, 0.74 ± 0.32 and 0.17 for ESS and EST (Table S2).

Population structure and LD decay

A total of 8192.04 Gb of raw reads were produced by sequencing, and 7493.03 Gb of clean reads were obtained by filtering. The Q20 values were ≥ 95.97%, the Q30 values were ≥ 92.56%, the sequencing depth was between 9.30 and 14.5, and the coverage was at least 1X ≥ 96.86%, indicating that the sequencing quality met the requirements for subsequent analysis. A total of 6,131,623 SNPs were found after SNP calling (Fig. 2A). Principal component analysis (PCA) of the Muscovy duck population revealed three groups and PC1 and PC2 were 1.37% and 1.2%, respectively, indicating that there was slight stratification (Fig. 2B). The distance of LD value decays from maximum to half is 284 bp, and the distance it decays to 0.1 is 118 kb (Fig. 2C).

GWAS analysis of egg quality

Screening of SNPs associated with egg weight

A total of 68 SNPs significantly related to EW were distributed on chromosomes (Chr) 2, 5, 9, 11, and 18 (Fig. 3A, Table S3). Significant SNPs were annotated to genes *proteasome assembly chaperone 4* (*PSMG4*), *solute carrier family 22 member 23* (*SLC22A23*), and *dynein axonemal heavy chain 5* (*DNAH5*) on chr 2; annotated to genes *fatty acid binding protein 6* (*FABP6*) on chr 9; annotated to genes *ADAM metallopeptidase with thrombospondin type 1 motif 17* (*ADAMST17*), *insulin-like growth factor 1 receptor* (*IGF1R*), and *neurotrophic receptor tyrosine kinase 3* (*NTRK3*) on chr 11; and annotated to genes *suppressor of cancer cell invasion* (*SCAI*) on chr 18 (Fig. 3A, B). Among them, densely significant SNPs appeared on chr 2 and chr 11, so LD analysis was conducted. The SNPs on chr 2 can be divided into three blocks corresponding to three genes, *PSMG4*, *SLC22A23*, and *DNAH5* (Fig. 3C). Because SNPs within each block are highly linked, a tagSNP can be selected to represent SNPs within the block. The SNPs 2_75684453_C > G, 2_76305509_A > G and 2_76350118_T > A were selected as tagSNPs for three blocks (Fig. 3D). For the SNP 2_75684453_C > G in block 1, the EW of the CC genotype was significantly greater than that of the CG and GG genotypes by 2.75 g ($P < 0.05$) and 5 g, respectively ($P < 0.01$), and the EW of the CG genotype was significantly heavier than that of the GG genotype by 2.25 g ($P < 0.01$, Fig. 3E). For the 2_76305509_A > G in block 2, the EW of AA and AG were significantly heavier than the GG by 4.14 g and 3.06 g, respectively ($P < 0.01$, Fig. 3F). For the 2_76350118_T > A in block 3, the EW of TT was 3.36 g and 5.47 g greater than the AA and TA ($P < 0.01$), respectively, and the TA genotype was 2.11 g greater than the AA ($P < 0.01$, Fig. 3G). SNPs distributed on chromosome 11 can be divided into three blocks corresponding to the *ADAMS17*, *IGF1R*, and *NTRK3* genes. The tagSNPs were 11_3834664_C > T, 11_4339778_C > T, and 11_8079686_C > T (Fig. 3H, I). For 11_3834664_C > T in block 1, EW of ducks with the TT genotype was 7.21 g and 5.15 g heavier than the CC and TC ($P < 0.01$), respectively, and the TC was 2.06 g heavier than the CC ($P < 0.01$, Fig. 3J). For 11_4339778_C > T in block 2, the

Table 1 Egg quality of muscovy Duck

	EW	SAD	LAD	ESI	AW	YW	AH	EST	ESS	YC
Mean	85.42	47.68	65.54	0.73	39.35	30.81	8.22	0.39	4.98	4.7
SD	5.31	1.38	1.97	0.03	4.63	2.53	0.92	0.05	0.95	0.7
CV (%)	6.22	2.89	3.01	3.69	11.77	8.22	11.14	11.58	19.14	15.08
Min	100.65	51.72	70.96	0.81	50.88	38.19	10.91	0.50	7.25	6
max	69.82	44.08	61.00	0.66	27.57	28.13	5.73	0.25	2.24	3
Heritability	0.19	0.16	0.15	0.11	0.16	0.27	0.01	0.25	0.21	0.41

Note: Egg weight (EW), egg albumen weight (AW), yolk weight (YW), long axis diameter (LAD) of the egg, short axis diameter (SAD), eggshell thickness (EST), egg shape index (ESI), yolk color (YC), and albumen height (AH)

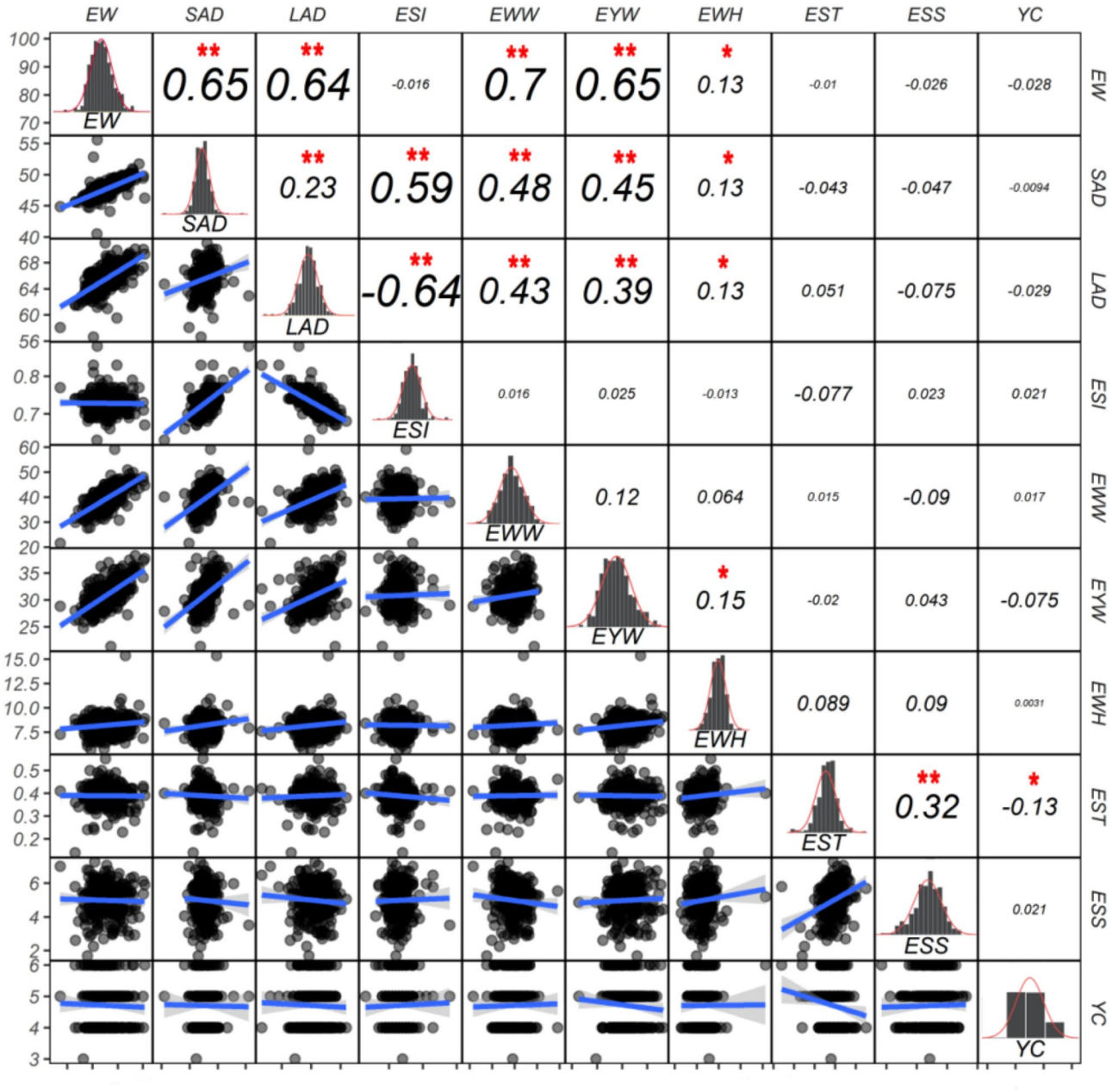

Fig. 1 Correlation analysis of egg quality in Muscovy ducks. The diagonal is the histogram of the trait distribution. The upper left corner shows the Pearson correlation coefficient between traits. * indicates $P < 0.05$, ** indicates $P < 0.01$. The lower left corner is the scatter plot of the correlation analysis. Weight of the egg (EW), albumen weight (AW), yolk weight (YW), long axis diameter (LAD) of the egg, short axis diameter (SAD), eggshell thickness (EST), egg shape index (ESI), yolk color (YC), and albumen height (AH) were measured

EW of TT and TC were significantly heavier than CC by 3.76 g and 2.86 g, respectively ($P < 0.01$, Fig. 3K). For 11_8079686_C > T in block 3, the EW of TT and TC were significantly heavier than CC by 4.4 g and 2 g, respectively, and TT was significantly heavier than TC by 2.4 g ($P < 0.01$, Fig. 3L).

Screening of SNPs associated with albumen weight

Thirteen significant SNPs associated with AW were distributed mainly on chr 1, 4, 12, 16, 21, and 30. SNPs of chr 12 were annotated to the gene *cingulin like 1* (*CGNL1*), and SNPs of chr 21 were annotated to *Sterile Alpha Motif Domain Containing 9 Like* (*SAMD9L*). The Q–Q results revealed that the P values associated with AW tended to be normally distributed, and significant P values could be

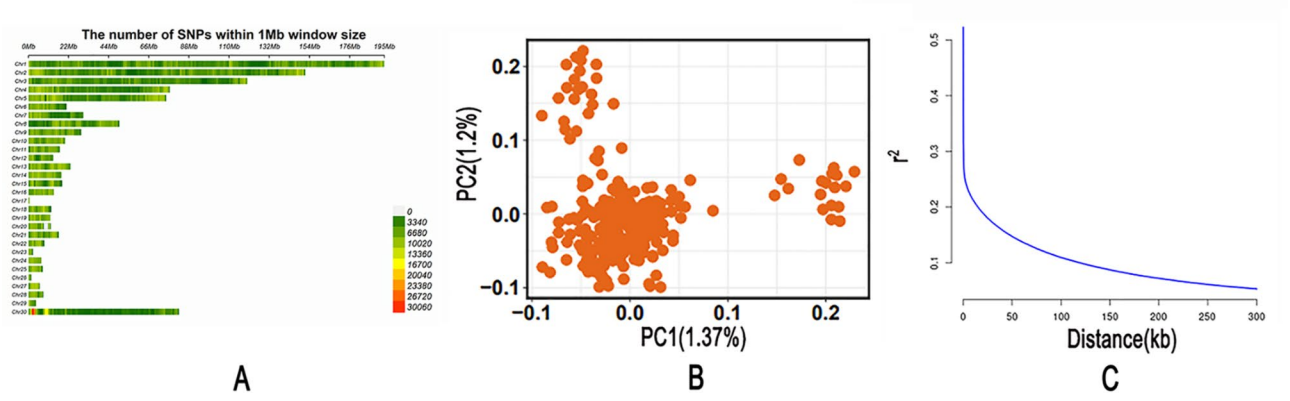

Fig. 2 Description of sequencing in Muscovy duck. (**A**) SNP distribution in each chromosome, (**B**) Population structure, and (**C**) LD decay. The R^2 value ranges from 0 to 1. "0" indicates that there is no linkage disequilibrium between the two loci, "1" indicates that a perfect linkage disequilibrium

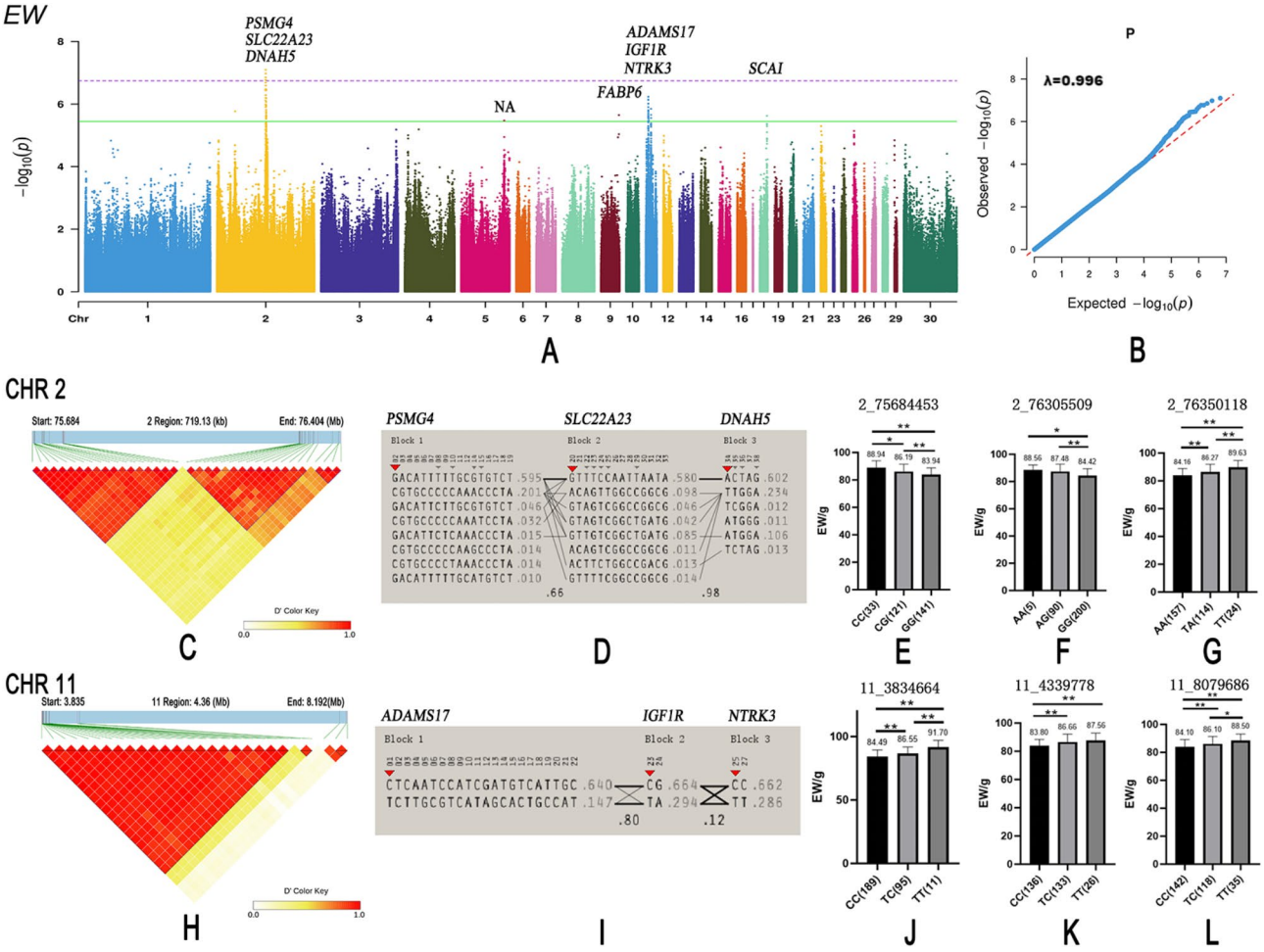

Fig. 3 Genes and tagSNPs related to egg weight (EW). (**A**) Manhattan plots of EW. (**B**) Quantile–quantile (Q–Q) plots of EW. (**C**) Linkage disequilibrium (LD) analysis of significant SNPs of the EW located on chromosome 2. (**D**) LD blocks of significant SNPs of the EW located on chromosome 2. (**E**–**G**) ANOVA of tagSNPs among different genotypes of the genes *PSMG4*, *SLC22A23*, and *DNAH5*. (**H**) LD analysis of significant SNPs of the EW located on chromosome 2. (**I**) LD blocks of significant SNPs of the EW located on chromosome 11. (**J**–**L**) ANOVA of EW at tagSNPs of the *ADAMS17*, *IGF1R*, and *NTRK3* genes. Chromosome 30 is the Z chromosome. TagSNPs are highlighted in red triangles

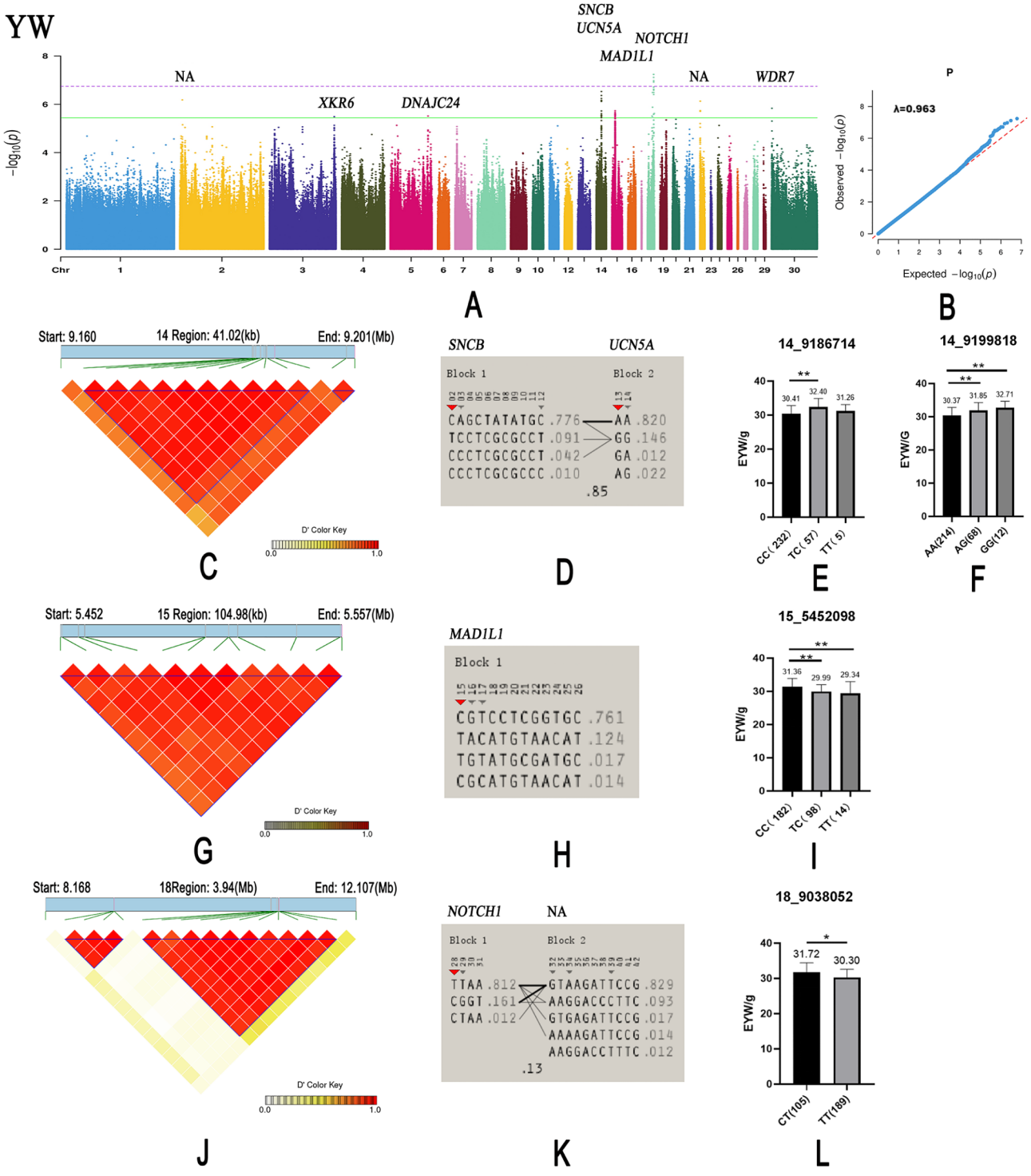

Fig. 4 Genes and tagSNPs related to egg yolk weight (YW). (**A**) Manhattan plots of the YW. (**B**) The Q–Q plots of YW. (**C**) LD analysis of significant SNPs located on Chr. 2. (**D**) LD blocks of significant SNPs located on Chr. 2. (**E**-**F**) ANOVA of tagSNPs among different genotypes of the *SNCB* and *UNC5A* genes. (**G**) LD analysis of significant SNPs located on chr 15. (**H**) LD blocks of significant SNPs located on chr 15. (**I**) ANOVA of tagSNPs of *MADIL1*. (**J**) LD analysis of significant SNPs located on chr 18. (**K**) LD blocks of significant SNPs located on chr 18. (**L**) ANOVA of tagSNPs of *NOTCH1*. Chromosome 30 is the Z chromosome. TagSNPs are highlighted in red triangles

false positives (Figure S1). Therefore, no further LD analysis was performed.

Screening of SNPs associated with yolk weight

Fifty significant SNPs associated with YW were distributed on chr 2, 3, 5, 14, 15, 18, 22, and 30, annotating genes *XK related 6* (*XKR6*, chr 3), *DnaJ heat shock protein family (Hsp40) member C24* (*DNAJC24*, chr5), *synuclein beta* (*SNCB*) and *Unc-5 Netrin Receptor A* (*UNC5A*, chr 14), *mitotic arrest deficient 1 like 1* (*MAD1L1*, chr 15), *notch receptor 1* (*NOTCH1*, chr 18), and *WD repeat domain 7* (*WDR7*, chr 30, Fig. 4A, B). Significant SNPs on chr 14 can be divided into two blocks, corresponding to two genes, *SNCB* and *UNC5A*, and two tagSNPs, 14_9186714_C > T and 14_9199818_A > G, respectively (Fig. 4C, D). For 14_9186714_C > T, the YW of the TC genotype was 1.99 g heavier than that of CC, and TT was less distributed in the population. For 14_9199818, the YW of GG and AG were 2.34 g and 0.86 g greater than that of the AA genotype (Fig. 4E, F). The significant SNPs on chr 15 were in one block, corresponding to the *MAD1L1* gene and tagSNP 15_5452098_C > T (Fig. 4G, H). For 15_5452098, the YW of CC was 1.37 g and 2.02 g greater than those of TC and TT, respectively ($P < 0.01$, Fig. 4I). Significant SNPs on chr 18 can be divided into two blocks: one block corresponds to the *NOTCH1* gene, and the tagSNP is 18_9038052_C > T, with a CT significantly greater than the CC by 1.42 g, the other has no gene annotated (Fig. 4J, K, L).

Screening of SNPs associated with the long-axis diameter, short-axis diameter, and egg shape index

Twenty-two significant SNPs associated with LAD were distributed on chr 1, 2, 4, 5, 6, 8, 11, and 30, which were annotated with the genes *BBX high mobility group box domain containing* (*BBX*, chr 1), *divergent protein kinase domain 1 A* (*DIPK1A*, chr 8), *zinc finger protein 423* (*ZNF423*, chr 10), and *haloacid dehalogenase like hydrolase domain containing 2* (*HDHD2*, chr 30), respectively. The Q–Q results revealed that the P values of the LAD tended to be normally distributed (Figure S1). Fifty-eight significant SNPs detected in SAD were distributed on chrs 1, 2, 3, 9, 13, 25, and 30, annotated genes *general transcription factor IIE subunit 1* (*GTF2E1*, chr 1) and *ankyrin repeat domain 33B* (*Ankrd33b*, chr 2), *SMAD family member 2* (*SMAD2*), *ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5* (*ST8SIA5*), *SET binding protein 1* (*SETBP1*), *nucleolar protein 6* (*NOL6*), *family with sequence similarity 219 member A* (*FAM219A*), *unc-13 homolog B* (*UNC13B*), and *RPTOR*

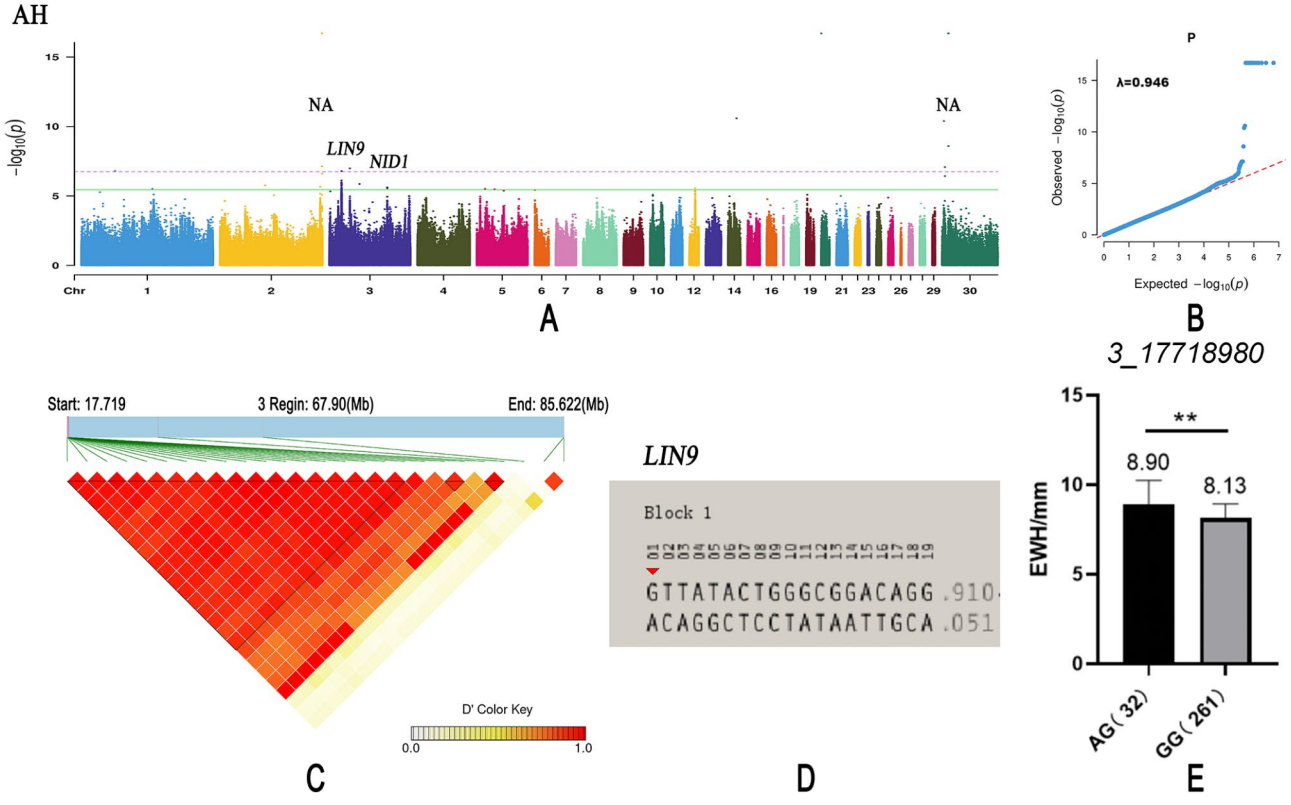

Fig. 5 Genes and tagSNPs related to albumen height (AH). (**A**) Manhattan plots of the AH. (**B**) The Q–Q plots of the AH. (**C**) LD analysis of significant SNPs located on Chr 3. (**D**) LD blocks of significant SNPs located on chr 2. (**E**) ANOVA of tagSNPs among different genotypes of LIN9. Chromosome 30 is the Z chromosome. TagSNPs are highlighted in red triangles

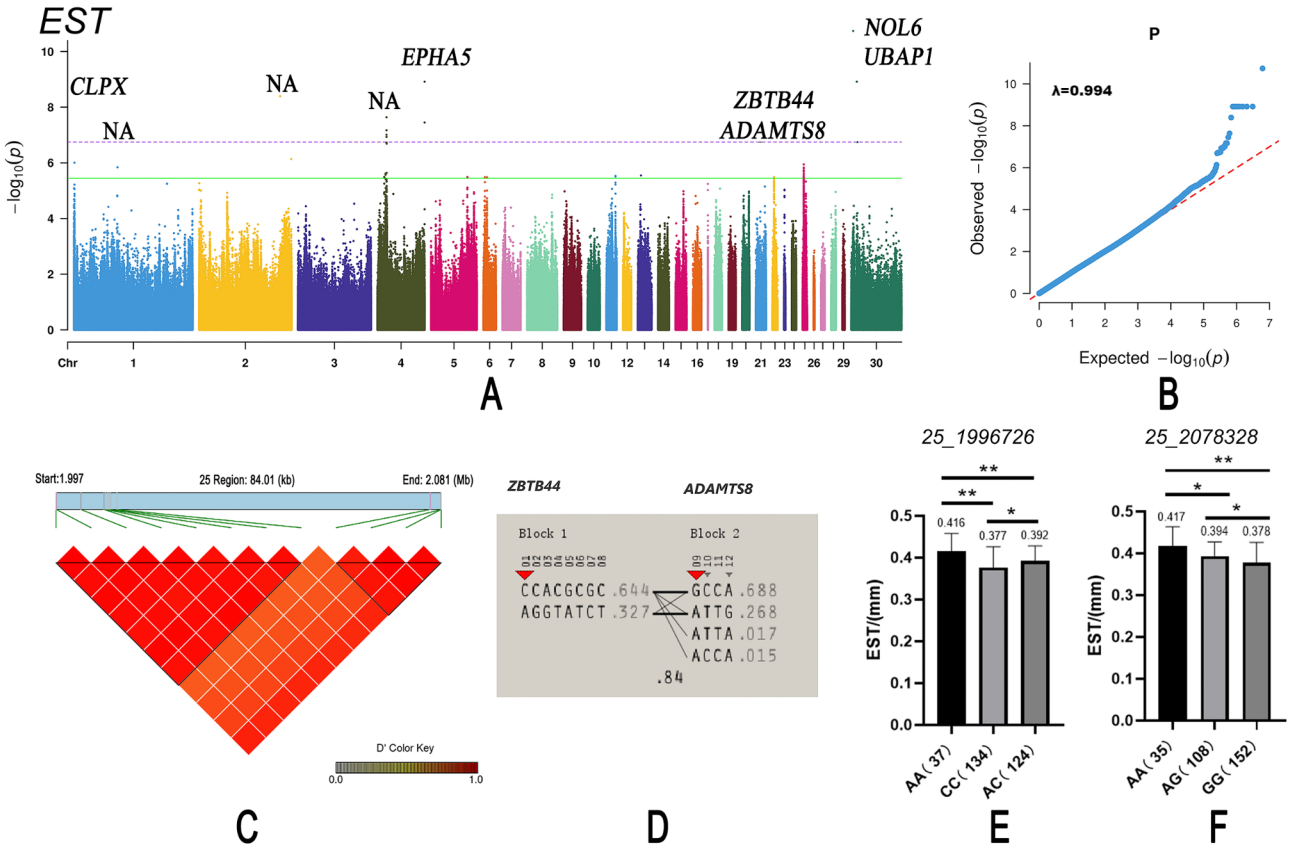

Fig. 6 Genes and tagSNPs related to eggshell thickness (EST). (**A**) Manhattan plots of the EST. (**B**) The Q–Q plots of the EST. (**C**) LD analysis of significant SNPs located on Chr. 25. (**D**) LD blocks of significant SNPs located on Chr. 25. (**E**-**F**) ANOVA of tagSNPs among different genotypes of the genes *ZBTB44* and *ADAMTS8*. Chromosome 30 is chromosome Z. TagSNPs are highlighted in red triangles

independent companion of MTOR complex 2 (*RICTOR*, chr30). Sixty-nine significant SNPs detected in ESI were distributed on chrs 1, 5, 7, 9, 13, 14, 15, 19, and 30. Significant SNPs in the chr30 annotated genes *SETBP1*, *NOL6*, and *FAM219A*. These genes were also annotated to significant SNPs associated with SAD. LD analysis revealed a low linkage between these significant sites, and the mutation frequency of these sites was too low; therefore, no further analysis was performed (Figure S2).

Screening of SNPs associated with albumen height

Fifty-four significant SNPs associated with AH were distributed on chrs 1, 2, 3, 5, 12, 14, 20, and 30. Significant SNPs on chr 3 annotated genes *lin-9 DREAM MuvB core complex component* (*LIN9*) and *nidogen 1 (NID1*, Fig. 5A, B). The LD analysis revealed that most SNPs were in the same block, corresponding to the *LIN9* gene, and the tagSNP was 3_17718980_A > G (Fig. 5C, D). The AH of the AG genotype was 0.77 mm greater than the GG genotype. The AA genotype individuals did not exist (Fig. 5E).

Screening of SNPs associated with eggshell thickness and eggshell strength

Fifty-one significant SNPs of the ESS were distributed on chrs 2, 5, 18, and 28, annotating the genes *clavesin 1* (*CLVS1*, chr 2), *NCK associated protein 5* (*KCKAP5*, chr 5), and *membrane palmitoylated protein 3* (*MPP3*, chr 28). The Q–Q results revealed that the *P* values of the ESS tended to be normally distributed (Figure S1).

Twelve significant SNPs of the EST were distributed on chr 1, 2, 4, 25, and 30, annotating the genes *caseinolytic mitochondrial matrix peptidase chaperone subunit B* (*CLPX*, chr 1), *EPH receptor A5* (*EPHA5*, chr 4), *A Disintegrin And Metalloproteinase With Thrombospondin Motifs 8* (*ADAMTS8*, *chr 25*), *zinc finger and BTB domain containing 44* (*ZBTB44*, chr 25), and *NOL6* and *ubiquitin associated protein 1* (*UBAP1*, chr 30, Fig. 6A, B). The significant SNPs on chr 25 were divided into two blocks, corresponding to the genes *ZBTB44* and *ADAMTS8*, and TagSNPs 25_1996726_A > C and 25_2078328_A > G, respectively (Fig. 6C, D). For 25_1996726_A > C, the AA genotype was 0.039 mm and 0.024 mm thicker than the CC and AC ($P < 0.01$), and the AC was 0.015 mm thicker than the CC ($P < 0.05$, Fig. 6E). For 25_2078328, the AA was 0.023 mm and 0.039 mm greater than the AG and

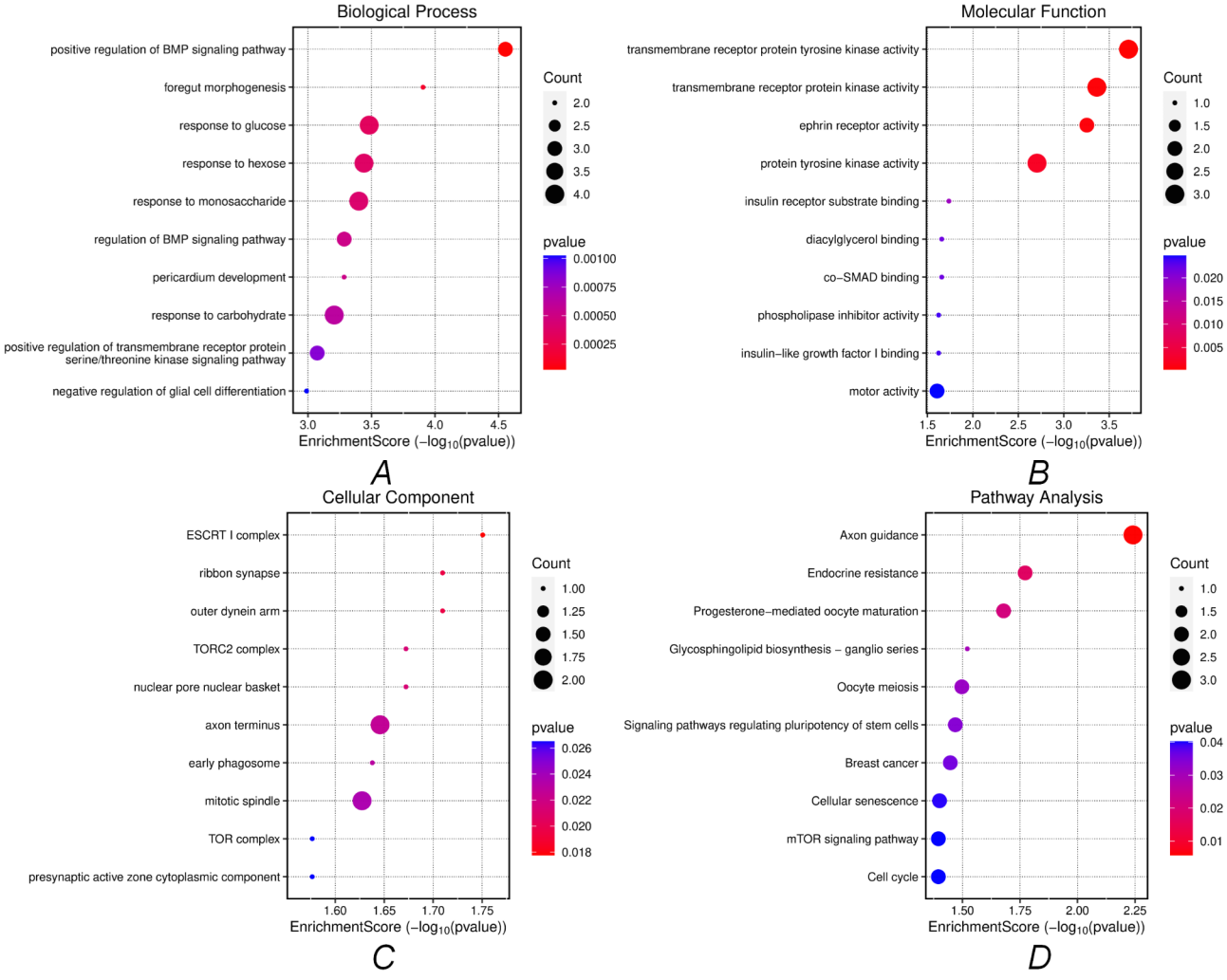

Fig. 7 GO and KEGG analyses of genes related to egg quality. (**A**) Dotplot of BP enrichment in GO analysis. (**B**) Dotplot of MF enrichment in GO analysis. (**C**) Dotplot of CC enrichment in GO analysis. (**D**) Dotplot of pathway enrichment in KEGG analysis

GG ($P < 0.01$), and the AG was 0.016 mm greater than the GG ($P < 0.05$, Fig. 6F).

Screening of SNPs associated with egg yolk color

Thirty significant SNPs of YC were distributed on chrs 1, 3, 4, 8, 9, and 16. Significant SNPs annotated the genes *ADAM metallopeptidase with thrombospondin type 1 motif 20* (*ADAMTS20)* on chr 1, annotated the genes *Ring Finger Protein 220* (*RNF220*) on chr 8, and annotated the genes *LIM domain kinase 2* (*LIMK2*) on chr 16 (Fig.S1). The Q−Q results revealed that the P values of YC tended to be normally distributed.

GO and KEGG enrichment analysis

To understand the functions of genes associated with Muscovy duck egg quality, GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses were conducted on these genes (Tables S4 and S5). For the biological process (BP) category, significant enrichment was found only in the process of positive regulation of the BMP signaling pathway (GO:0030513, Fig. 7A, Table S4). For the molecular function (MF) category, significant enrichments were mainly concentrated in transmembrane receptor protein tyrosine kinase activity (GO:0004714), ephrin receptor activity (GO:0005003), and protein tyrosine kinase activity (GO:0004713), et al. (Fig. 7B). No significant enrichments were detected in the cellular component (CC) category (Fig. 7C). For the KEGG analysis, significant enrichments were observed in pathways such as Axon guidance (hsa04360), Endocrine resistance (hsa01522), and Progesterone - mediated oocyte maturation (hsa04914), et al. (Fig. 7D).

ROH analysis

A total of 38,113 ROH regions were identified in 295 individuals through ROH analysis. Each individual had, on average, 129 ROH regions, and most individuals had between 119 and 131 ROH regions (Fig. 8A). The average

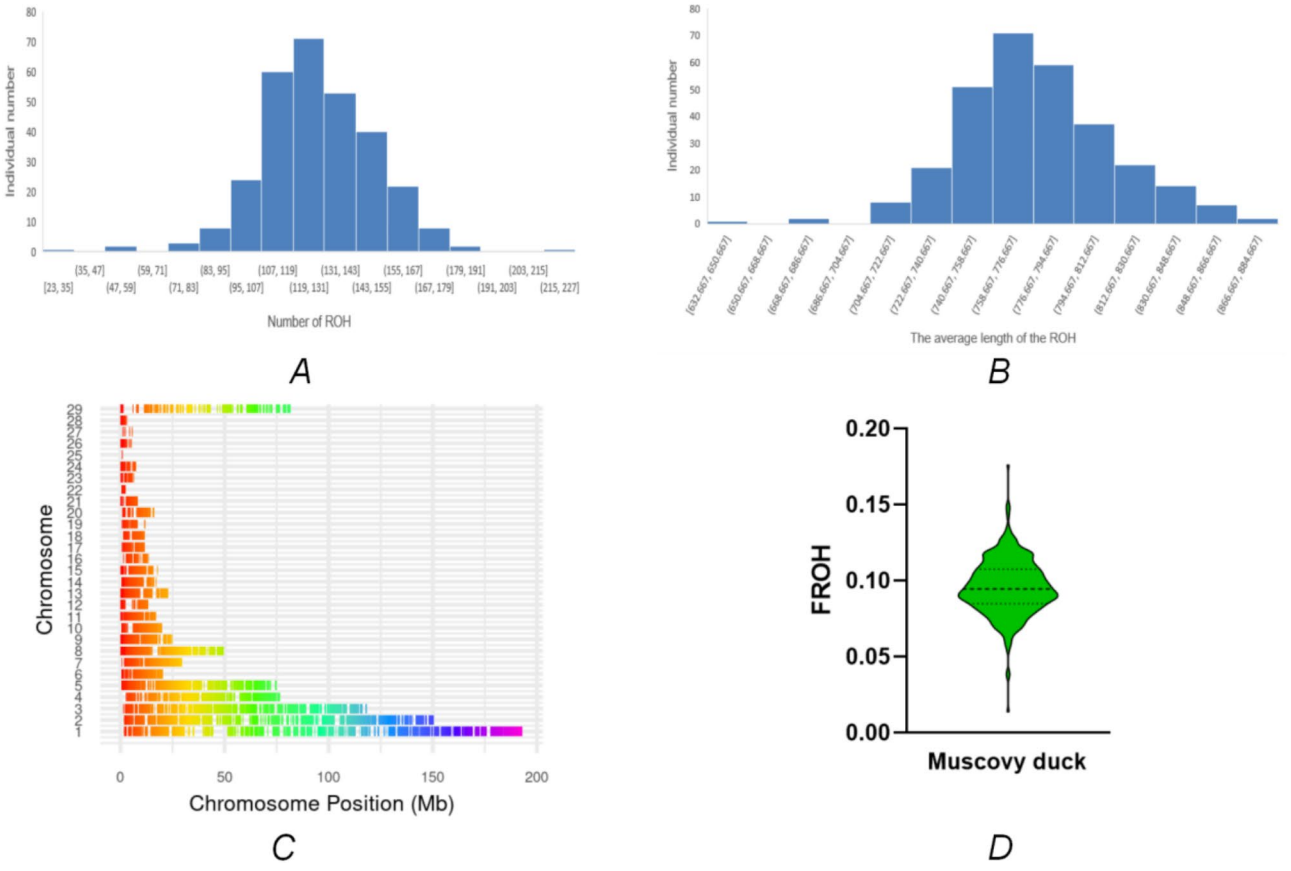

Fig. 8 Runs of Homozygosity (ROH analysis of Muscovy duck). (**A**) Distribution of ROH among individuals. (**B**) Average length distribution of ROH. (**C**) Distribution of ROH in different chromosomes. (**D**) Genomic inbreeding coefficient based on Runs of Homozygosity (FROH) of the Muscovy duck population

total length of ROHs per individual was 100,821 kb, and the average length of each ROH was 778 kb (Fig. 8B). The average length of ROHs in most individuals ranged from 758 to 776 kb. The chromosomal distribution of ROHs is shown in Fig. 8C. The inbreeding coefficient (FROH) of the Muscovy duck population in this study was calculated to be 0.096 (Fig. 8D). There was no positional overlap between the hotspots identified in ROH analysis (Table S6) and the significant SNPs associated with egg quality.

Discussion

Compared with other native Chinese duck breeds, such as Longyan duck [31], Guangxi small-hemp duck [32], Shaoxing duck [33], Putian black duck [34], and Jinding duck [35], Muscovy duck has heavier EW and YW. Exceptionally, EW of Muscovy duck is less than that of Pekin ducks [36], while the YW was higher than that of Pekin duck, resulting in a higher yolk ratio [37]. Compared with these duck breeds, Muscovy duck also has a higher ESS [31–37].

Due to their relatively fast growth rate, the time they spend in the eggshell accounts for a relatively large proportion of the period from hatching to market. Compared with Peking duck, which spend time in the eggshell account for 44% of the time from hatching to market [37], Muscovy ducks spend 33% of their time in their eggshells. On the other hand, the qualified eggs of meat poultry are almost all used for hatching, and when the qualified egg rate and hatching rate are improved through breeding, the cost of each duckling will be decreased. It is obvious that egg quality has a significant effect on hatchability and post-hatching, however, the specific details remain to be further studied [7, 9].

The egg quality of most poultry has moderate heritability (0.2–0.4) and can be effectively improved by generational selection [37]. Similarly, the heritability of egg quality traits in Muscovy duck was also moderate, suggesting that egg quality could also be selected through normal or molecular breeding. Evidence has shown that one generational selection has effectively increased the yolk ratio in White Leghorns [38]. The low LD decay distance and inbreeding coefficient of 0.096 indicated high recombination and low inbreeding of the tested Muscovy duck group. The average R^2 of all SNPs in Jinling White ducks was 0.24, and the R^2 of 0.2 was 30 kb [39]. The maximum R2 of the F2 segregating population of

Chinese Crested and Cherry Valley ducks was 0.582, and the distance of LD decay to "$R^2 = 0.1$" was 80,977 bp [40]. A rapid LD decay commonly been observed in poultry populations might due to their short generation interval.

The BMP signaling pathway plays an important role in ovarian function and follicle development processes [41]. Among the genes enriched in this pathway, is *RNF423*, which belongs to the same family as *RNF111* whose harmful mutations have been proven to lead to premature ovarian failure in mice and humans due to the ability of the ubiquitin ligases they encode to regulate the BMP pathway [42]. Another member of this family, *RNF220*, was also found to be related to egg quality in this study. The enriched KEGG pathways, which involve genes of *UNC5A*, *EPHA5*, *LIMK2*, *IGF1R*, *NOTCH1*, and *MAD1L1*, are associated with neural development and reproductive hormones. *IGF-1* and *IGF-1R* can activate granulosa cells to secrete progesterone in laying hens and bovines [43–45]. The SNPs of *IGF1R* in quail (Coturnica japonica) are associated with EW, yolk width, shell thickness, egg length, and egg diameter [46]. *IGF1R* was also found to be correlated with EW in this study. Several other genes are associated with ovarian function and development. *MAD1L1* is involved in cell division, and abnormal expression of this gene can lead to ovarian cancer [47, 48]. *NOTCH1*, a member of the Notch family, plays a significant role in both ovarian follicle number and lipid metabolism, with its downregulation reducing the number of ovarian follicles [49, 50]. *EPHA5*, a component of the Ephrin/EPH signaling pathway, interacts with the WNT signaling pathway during crucial biological processes such as embryogenesis, tissue regeneration, and carcinogenesis [51]. Studies have shown an association between *EPHA5* and obesity in mice [52].

In addit ion, among the genes identified in this study, some have been reported in previous research to be associated with egg quality or ovarian development. *SLC22A23* was significantly associated with EW in this study, and its family member, *SLC5A7*, was also associated with EW in Lingkun chickens [53]. It is a transmembrane transporter that has been found in pigs to be associated with foraging behavior and liver fat deposition [54]. *WDR7*, a protein that contains the WD repeat domain, was found to be associated with YW in this study. GWAS results indicated that among other genes with this domain, *WDR76* was related to yolk moisture content and *WDR48* was related to the HU [55, 56]. *WRD7* is associated with type 2 diabetes mellitus and participates in lipid metabolism in humans [57]. *NTRK3* was associated with EW in this study. Moreover, it has also been found that it is associated with the weight of yellow-feathered broilers and high egg production in white Leghorn chickens [58, 59]. Besides, *NTRK3* can promote ovarian primordial to primary follicle transition [60]. *NOL6* was associated with EST in this study, and a genome-wide selection sweep analysis of various duck breeds, including the Jinding duck, Shanma duck, Youxian Partridge duck, and Taiwan Brown tsaiya duck, suggested that *NOL6* may be associated with egg production in egg-laying ducks [61].

The genetic control of egg quality traits is intricate, posing challenges in elucidating how these genes influence variations in egg quality. Nonetheless, the identification and typing of specific SNPs associated with egg quality traits are straightforward, practical, and cost-effective endeavors. Through GWAS analysis, several SNPs and genes related to EW, YW, AH, and ESS in Muscovy ducks were identified. Despite the quality control and threshold setting, there is still the possibility of false positive results due to the limited sample size, which needs to be proven by further tests.

Conclusion

In conclusion, we found some possible candidate genes related to egg quality of Muscovy duck through GWAS. The genes associated with EW were *PSMG4*, *SLC22A23*, *DNAH5*, *FABP6*, *ADAMST17*, *IGF1R*, *NTRK3*, and *SCAI*. The genes associated with YW were *XKR6*, *DNAJC24*, *SNCB*, *UNC5A*, *MAD1L1*, *NOTCH1*, and *WDR7*. The genes associated with AH were *LIN9* and *NID1*. The genes associated with ESS were *CLPX*, *EPHA5*, *ZBTB44*, *NOL6*, and *UBAP1*. Moreover, some tagSNPs were screened via LD analysis.

Abbreviations

EW	Egg weight
AW	Albumen weight
YW	Yolk weight
LAD	Long axis diameter
SAD	Short axis diameter
EST	Eggshell thickness
ESI	Egg shape index
YC	Yolk color
AH	Albumen height
chr	chromosome
LD	Linkage disequilibrium

Supplementary Information

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Supplementary Material 1 Fig. S1

Supplementary Material 2 Fig. S2

Supplementary Material 3: Table S1. Phenotypic information of Muscovy duck egg quality.

Supplementary Material 4: Table S3. Significant SNPs and their annotation information correlated with Muscovy duck egg quality.

Supplementary Material 5: Table S4. GO (Gene Ontology) analysis of genes related to Muscovy duck egg quality.

Supplementary Material 6: Table S5. KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis of genes related to Muscovy duck egg quality.

Supplementary Material 7: Table S6. The hotspots in the runs of homozygosity (ROH) analysis of Muscovy ducks.

Supplementary Material 8: Table S2. Genetic correlation and phenotypic correlation among quality traits of Muscovy duck eggs.

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Author contributions
XY and CZ conceived of the study and provided fnancial support. SQ performed the investigation, performed the experiment and data collection. WL and LP analyzed the data, and WL wrote the draft. LP, DY, WH, XQ and HQ participated in sample collection. All authors reviewed and agreed to the published version of the manuscript.

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Data availability
The datasets generated and/or analysed during the current study are available in the Genome Variation Map in National Genomics Data Center repository, [https://bigd.big.ac.cn/gvm/getProjectDetail?Project=GVM000989, accession number is GVM000989.]

Declarations

Ethics approval and consent to participate
The animal experiment was reviewed and approved by the Institutional Animal Care and Use Committee of the Anhui Agricultural University (No. SYDW-P20200600601). The experiments were performed according to the Regulations for the Administration of Affairs Concerning Experimental Animals and the Standards for the Administration of Experimental Practices, as well as the ARRIVE guidelines version 2.0. This study has obtained informed consent from the Anhui Yongqiang Co., LTD.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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