

Genome-Wide Association Study to Identify SNPs and Haplotypes Associated with Reproductive Performance and Egg Quality Traits in Geese

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Research

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Abstract

Background: Geese are one of the most economically important waterfowl worldwide. However, suboptimal reproductive performance hinders the development of the goose rearing industry. With the increasing public concern surrounding animal health and the regulation of the goose rearing industry, reproductive performance and egg quality have become a major focus for researchers. To promote the accuracy of selective breeding and identify important candidate genes associated with reproductive performance and egg quality traits, we examined the birth body weight (BBW), egg number at 48 weeks (EN48), egg number at 60 weeks (EN60) and egg yolk color (EYC) among 209 Sichuan White geese using a genome-wide association study (GWAS).

Results: In this study, we obtained 2.896 Tb of raw whole-genome sequencing data with a mean depth coverage of 12.44. A total of 9,279,339 single nucleotide polymorphisms (SNPs) were identified in the goose genome. The GWAS analysis showed that 26 SNPs were significantly associated with the examined birth body weight (BBW), egg number in 48- and 60-week-old geese (EN48 and EN60) traits and egg yolk color (EYC), respectively. Notably, two genomic regions, a 28.85 kb genomic region (4512855bp ~ 4541709bp) on chromosome 35, and 294.57kb genomic region (21069009 b ~ 21363580 bp) located on chromosome 5, were significantly associated with egg number in 48- and 60-week-old geese (EN48 and EN60) egg yolk color (EYC), respectively. In addition, we performed the MALDI-TOP MS method to detected SNPs and haplotypes markers for EN48, EN60 and EYC traits were identified in the candidate gene *TMEM161A*, *CALCR*, *TFPI2*, and *GLP1R*, respectively

Conclusions: The SNPs, haplotypes markers and genes identified in this study can be used for marker-assisted selection of reproductive performance and egg quality traits in geese, promoting the accuracy of selective breeding. In addition, the identified candidate genes significantly associated with the traits provide a foundation for a better understanding of the mechanisms underlying reproduction and egg quality in geese.

Background

As one of the most economically important waterfowl worldwide, geese are widely reared for their eggs, meat, livers, feathers, and other by-products [1]. Geese are seasonal breeding waterfowl, meaning that reproduction is stimulated or inhibited by light signals, resulting in a non-laying period during the reproductive cycle [2, 3]. The broodiness characteristic of some geese further decreases overall egg production, hindering the development of the goose industry [4].

Eggs are an excellent and inexpensive protein source [5]. The yolk is the nutrient-bearing portion of an egg and contains a rich array of amino acids, fatty acids, vitamins, and minerals, the fundamental purpose of which is to provide nutrition for the developing embryo [6, 7]. The shell is also an essential component, with important physiological roles in protection (of the egg contents), respiration, and provision of calcium ions for embryonic development [8]. In addition, the shell can affect critical economic indicators

such as embryo viability, hatchability, egg production, and breakage rate [9–11]. With the increasing public concern surrounding animal health and the regulation of the goose industry, reproductive performance and egg quality have become a major focus for researchers [12, 13].

In birds, reproductive performance and egg quality are quantitative traits, which, as well as having genetic determinants, are also influenced by diet, housing, environment, management, disease, and other factors [13]. Genome-wide association studies (GWAS) are used to detect genes or genetic variations associated with specific traits [14]. To date, various genes have been associated with reproductive performance, egg quality, and other economically important traits in poultry. Once validated, these loci can be used for marker-assisted selection to significantly improve economic value and selective breeding techniques [15–17]. However, little is known about genes and markers associated with reproduction performance and egg quality traits in geese.

In the current study, we used a whole-genome resequencing approach to identify single nucleotide polymorphisms (SNPs) in the goose genome. We then conducted a genome-wide association analysis of egg laying and egg quality traits to identify the corresponding genomic regions and candidate genes, laying the foundation for further study of the traits to accelerate goose research and genetic improvement.

Methods

Ethics statement

All experiments involving animals were performed according to the laws and regulations established by the Ministry of Agriculture of China (Beijing, China). The study was approved by the Animal Care and Welfare Committee of the Chinese Chongqing Academy of Animal Science (Chongqing, China).

Experimental animals and phenotypic traits

A total of 209 Sichuan White geese were used in this study. Each bird was reared from birth to the non-laying period (65 weeks). All individuals were housed in single cages (600 × 800 × 900 mm) at the AnFu waterfowl-breeding base in Chongqing City, China (105.478°N, 29.343°E). All geese were fed the same diet with free access to water under natural temperatures and artificial lighting (12 h of light per day). At week 28, blood samples were collected from the wings of all 209 geese using vacuum tubes containing ethylenediaminetetraacetic acid and used for DNA extraction.

During the egg laying period (weeks 28–64), we collected and marked eggs from each goose daily. The birth body weight (BBW), egg number at 48 weeks (EN48), and egg number at 60 weeks (EN60) were recorded for each goose. At weeks 35 to 40, three consecutive eggs were collected from each individual and assessed for egg yolk color (EYC).

Whole-genome resequencing and SNP calling

DNA was extracted from the whole blood samples using a genomic DNA extraction Kit (DP332; Tiangen Biotech, Beijing, China). DNA concentration and quality were determined using a Nano Vue spectrophotometer (Cytiva Life Sciences, Marlborough, MA, USA) and agarose gel electrophoresis to fulfill the requirements for the Illumina sequencing platform.

Whole-genome resequencing data were obtained using the Illumina HiSeq X Ten platform (Illumina, San Diego, CA, USA). After read filtering, high-quality reads were mapped to a goose complete genome sequence assembled by our lab (data not shown) using BWA software [18]. SNP calling was conducted using GATK software [19].

Genotyping and quality control

Quality control was conducted at both the individual and SNP levels. At the individual level, only samples with genotype data that were $\geq 95\%$ complete were included in the trait's phenotype dataset. SNPs were filtered using Plink [20] according to the following criteria: Hardy-Weinberg equilibrium value $> 10^{-7}$, minor allele frequency > 0.05 , and proportion of missing genotypes < 0.05 . In addition, individuals with $< 10\%$ of genotypes missing were retained. Following quality control, SNPs identified from the 209 individuals were used for principal component analysis using the model implemented in Plink.

Genomic prediction

The identified traits and SNPs were then used to conduct a GWAS using a mixed linear model in the Genome-Wide Efficient Mixed Model Association (GEMMA) software package [21] using the following statistical model: $y = W\alpha + x\beta + \mu + \varepsilon$, where y is the reproductive phenotype or egg quality value for all individuals, W is a covariance matrix used to control population structure, α is a vector of corresponding coefficients, including the intercept, x is the genotype of the SNP or haplotype marker, β is the effect size of the SNP or haplotype marker for the phenotypes, μ is a vector of random polygenic effects, and ε is a vector of random residuals. The Wald test statistic was used to calculate the significance of the SNPs and phenotypes, while the association analysis results were corrected using Bonferroni's method.

The genotypes of the significantly associated SNPs identified in the GWAS were determined by iPLEX matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOP MS) using the MassARRAY genotyping platform (Agena Bioscience, San Diego, CA, USA). Amplification and extension primers (**Table S1**) were designed to amplify the target sequences and hybridize and elongate the fragments at the nucleotide position of interest, respectively.

For all of the traits, Manhattan plots were generated using the GAP package (<https://cran.r-project.org/web/packages/gap/>) and quantile-quantile (QQ) plots were drawn using the qqman package (<https://cran.r-project.org/web/packages/qqman/>), both in R project software. We used the GenABEL package to calculate the extent of false-positive signals in the results using the genomic inflation factor (λ) [22].

SNP-based haplotype blocks

Association analysis of the SNPs and haplotypes and phenotypic traits was carried out using a general linear model in JMP version 13.0 using the same equation: $y = W\alpha + x\beta + \mu + \varepsilon$. Haplotype blocks and SNPs were separately estimated for every trait. The least-squares mean of each genotype or haplotype block was then calculated, and differences between the genotypes were analyzed using the Bonferroni test.

SNP annotation

BEDTools software[23] was used to extract genetic information from 500-kb regions upstream and downstream of each potential SNP in the goose genome, while SNP annotation was conducted using Annovar software[24] (SnpEff, Annovar, VEP, oncotator). The detected genes were then searched against the chicken and human genomes using BioMart software (<http://asia.ensembl.org/biomart/>) to identify homologous genes, while gene function annotation was carried out using the metascape website[25] (<https://metascape.org/gp/index.html#/main/step1>).

Results

Description of phenotypic traits

The body weight, egg laying, and egg-quality traits of all 209 Sichuan White geese were recorded throughout the laying period and are summarized in **Table 1**. The reproduction performance BBW, EN48, EN60 and EYC traits confirmed that Sichuan White geese are a high yield, medium body-sized breed. The egg laying traits of the study population were consistent with those described previously for the breed.

Whole-genome resequencing and SNP calling

We generated 2.896 Tb of raw whole-genome resequencing data from 209 female Sichuan White geese, with a Mean depth coverage of 12.44. After filtering, a total of 2.891 Tb of high-quality sequence data were mapped to a goose genome sequence, with an average mapping rate of 98.15% (96.58%–98.38%) (**Table S2**). The average GC content was 42.85%, which is consistent with that previously reported for the goose genome [26-28]. A total of 9,279,339 SNPs identified in goose genome were used for further analysis (**Figure S1**).

Genome-wide association study

Principle component analysis revealed slight stratification within the goose population (**Figure 1**). Therefore, we used a mixed linear model in the GEMMA software package to adjust for variations in population structure and conduct a GWAS using the selected traits. In total, 26 SNPs were found to be significantly associated with the traits (**Table 2**), located within 14 genes surrounded these regions (**Table 2**). The Manhattan and QQ plots for the traits are shown in **Figure 2**.

We performed the functional enrichment analysis the egg number traits (EN48 and EN60) and EYC traits, respectively. The 37 Gene Ontology terms were significantly enriched ($p < 0.05$) for EN48 and EN60 traits, such as “response to radiation (GO:0009314)”, “response to light stimulus (GO:0009416)”, and “cellular response to hormone stimulus (GO:0032870)” (**Table S3, Figure 3**). The 21 Gene Ontology terms were significantly enriched ($p < 0.05$) among the genes for EYC trait, including “regulation of epidermal growth factor-activated receptor activity” (GO:0007176), “regulation of signaling receptor activity” (GO:0010469) and “regulation of epidermal growth factor receptor signaling pathway” (GO:0042058) (**Table S4, Figure 3**).

Haplotype-based association analyses with EN48, EN60 and EYC traits

Haplotypes were constructed for the regions containing the significantly associated SNPs. We found that 5 SNPs in *TMEM161A* (transmembrane protein 161A), located within a 28.85 kb genomic region (4512855bp ~ 4541709bp) of chromosome 35, were significantly associated with EN48 and EN60 (**Figure 4A**). The 5 SNPs and genotypic distributions in goose were statistically analyzed, and associations between genotypes and EN48 and EN60 traits were determined (**Table 3, Figure 4A**). Haplotype association analysis confirmed that the corresponding haplotype for this region was significantly associated with both the EN48 and EN60 traits. Individuals with the genotype (CCAAAGAA) produced minimal eggs at 48 weeks and 60 weeks compared with individuals with the other genotypes (**Table S5**).

On chromosome 5, a haplotype within a 294.57kb genomic region (21069009 b ~ 21363580 bp) located on chromosome 5, was constructed based on 5 SNPs (**Figure 4B, Table 3**) in the genes *GLP1R*, *CALCR*, and *TFPI2* that were significantly associated with EYC. The 5 SNPs and genotypic distributions in goose were statistically analyzed, and associations between genotypes and EYC traits were determined (**Table 3**). Haplotype association analysis showed that the haplotype was also significantly associated with EYC, and that there were significant differences among genotypes. For example, individuals with the genotypes (AGCTGAGAGT) and (GGTTGGAAGG) showed significantly higher and lower EYC values, respectively, than individuals with other genotypes (**Table S5**).

Discussion

Sichuan White is a popular Chinese goose breed reared for both meat and eggs. While egg laying and egg quality traits have been studied intensively in avians, including chickens[29, 30], ducks[15, 31], and quail[32], few studies have examined traits other than those associated with egg laying in the goose genome [13].

In the current study, a GWAS using 209 geese identified 27 SNPs significantly correlated with BBW, EN48, EN60 and EYC traits. As one of the most economically important traits, BBW is a major focus of goose breeding and has been correlated with a number of other performance traits in birds[33]. A SNP located within *SMAP2* (stromal membrane-associated protein 2) was significantly associated with BW. In mammals, *SMAP2* is necessary for formation in spermiogenesis[34], with *SMAP2*-deficient mice

displaying male infertility[35], globozoospermia[36], asthenozoospermia[36], and abnormal acrosome formation[36]. Therefore, *SMAP2* may be essential for reproduction in geese, regulating the BBW trait.

It is noteworthy that four SNPs situated within *TMEM161A* were grouped into a haplotype block, and association analyses confirmed that both the SNPs and the haplotype were significantly associated with EN48 and EN60. *TMEM161A*, also known as an adaptive response to oxidative stress protein 29 (*AROS-29*), is involved in several cellular processes, including the adaptive response to oxidative stress [37], the response to ultraviolet radiation, and the response to retinoic acid, and is a negative regulator of the intrinsic apoptotic signaling pathway in response to DNA damage and a positive regulator of the DNA repair response. In birds, retinoic acid promotes embryo development and egg production and can regulate egg number [38, 39]. Sichuan White geese display seasonal reproduction as an adaptation to migration habit of the domesticate from wild goose and are therefore sensitive UV radiation. Because *TMEM161A* plays a key role in the response to UV light[40], it is possible that the artificial selection pressures resulting from the intensification of animal production systems contributed to the appearance of the four SNPs in *TMEM161A*, satisfying the demand for increased egg production and aiding in adaptation to the production environment. Mover, we also identified the other 5 candidate genes for the EN48 or EN60 traits, *PGM2L* (Glucose 1,6-bisphosphate synthase), *DP13B* (DCC-interacting protein 13-beta), *DP13B* (DCC-interacting protein 13-beta), *RTJK* (RNA-directed DNA polymerase from mobile element jockey), *S2542* (Mitochondrial coenzyme A transporter SLC25A42). Previous studies have identified many candidate genes corresponding to reproductive traits in geese [41]. However, the positional candidate genes for egg number identified in the current study did not overlap with these genes. The egg number trait in poultry is quantitative and is regulated by numerous genes as well as the environment[42]. The positional candidate genes and SNPs identified here were all associated with egg production and micro-effect control of the examined traits. In addition, previous studies have examined a range of different breeds raised under diverse conditions, resulting in differences in the artificial selection pressures among studies that would undoubtedly influence egg number. Further, while the population sizes used in these studies were sufficient to draw goose breed-specific conclusions, they were not large enough to make species-wide predictions.

The biology pathway analysis of the candidate genes for EN48 and EN60 highly enrich in the response to radiation (GO:0009314), response to light stimulus (GO:0009416), cellular response to hormone stimulus (GO:0032870), response to and peptide hormone (GO:0043434) (Table 3). As we known, goose is the seasonal breeding goose were regulated by the light and the reproduction actives were controlled by the cycling reproduction hormones. We speculated that the candidate genes for EN48 or EN60 were response to the light or the reproduction hormones, involving in to regulate the reproduction activities, the mechanism needed to be further studied.

EYC is a crucial economic egg quality character because consumers associate yolk color with nutrition in [43]. Studies have shown that EYC is affected by genetic [44], environmental [45], housing [46], and dietary [47] factors. In the current study, twelve SNPs were significantly associated with EYC in Sichuan White geese. It is worth noting that five of these SNPs were constructed into a haplotype, resulting in the

identification of positional candidate genes *CALCR* (calcitonin receptor), *TFPI2* (tissue factor pathway inhibitor 2), and *GLP1R* (glucagon-like peptide 1 receptor). *CALCR* is a receptor that binds the peptide hormone calcitonin, and is involved in calcium homeostasis, bone formation and metabolism, and lipid metabolism[48, 49]. *GLP1R*, a member of the glucagon receptor family of G protein-coupled receptors, is involved in the control of blood sugar levels by enhancing insulin secretion[50]. Interestingly, *CALCR* and *GLP1R* regulate peptides that have anorexic effects, suppressing long-term food intake and promoting significant weight loss [51, 52]. *TFPI-2* belongs to the Kunitz-type family of protease inhibitors and regulates matrix metalloproteinase activation and extracellular matrix degradation. Deficiency in this gene in mice accelerate the development of atherosclerotic lesions[53]. In addition, over-expression of *TFPI-2* strongly inhibits the proliferation and migration of vascular smooth muscle cells, and affects smooth muscle cell proliferation and migration [53, 54]. Therefore, these positional candidate genes may control the EYC trait by affecting food intake or lipid metabolism in geese. EYC is a quantitative trait in birds and is regulated by many genes in poultry[55]. In agreement with previous studies, we also identified multiple other positional candidate genes corresponding to the twelve SNPs, including *MBB1A* (Myb-binding protein 1A-like protein), *CB042* (uncharacterized protein C2orf42), *CACB2*, (voltage-dependent L-type calcium channel subunit beta-2), *CCM2* (cerebral cavernous malformations protein 2 homolog), and *ZBT46* (zinc finger and BTB domain-containing protein 46), the effects of which on EYC require further study.

The GO pathway analysis revealed that the regulation of epidermal growth factor-activated receptor activity (GO:0007176), regulation of epidermal growth factor receptor signaling pathway (GO:0042058) was remarkably enriched for EYC traits in goose. the two GO terms modulate the EGF-activated receptor activity. In mammal or birds, EGF-like growth factors play an important role in the ovulatory follicle[56], maturation of the cumulus-oocyte complex[57], oocyte maturation and development[58]. One of the key factors affected the EYC were genetic[59]. We propose that the candidate genes for EYC traits the EGF-activated receptor factors, might regulated the process of oocyte maturation and development, and determined the EYC traits in goose.

Conclusions

We predicted 26 candidate SNPs for the reproduction performance and egg quality traits in Sichuan white goose. Furthermore, The SNPs and haplotypes markers for EN48, EN60 and EYC, were identified in the candidate gene *TMEM161A*, *CALCR*, *TFPI2*, and *GLP1R*, respectively. The SNPs genotypes and SNPs haplotypes could be the markers for the egg production and EYC in the artificial selection procession in Sichuan White goose.

List Of Abbreviations

AROS-29

oxidative stress protein 29; BBW:birth body weight; CACB2:voltage-dependent L-type calcium channel subunit beta-2; CALCR:calcitonin receptor; CB042:uncharacterized protein C2orf42; CCM2:cerebral

cavernous malformations protein 2 homolog; EN48:egg number at 48 weeks; EN60:egg number at 60 weeks; GWAS:genome-wide association study; GEMMA:Genome-Wide Efficient Mixed Model Association; GLP1R:glucagon-like peptide 1 receptor; LD:Linkage disequilibrium; MALDI-TOP MS:matrix-assisted laser desorption-ionization time-of-flight mass spectrometry; MBB1A:Myb-binding protein 1A-like protein; QQ plot:quantile-quantile plots; SNPs:single nucleotide polymorphisms (SNPs); SMAP2:stromal membrane-associated protein 2; SRA:Short Read Archive; TMEM161A:transmembrane protein 161A; TFPI2:tissue factor pathway inhibitor 2; TFPI2:tissue factor pathway inhibitor 2; ZBT46:zinc finger and BTB domain-containing protein 46.

Declarations

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Availability of data and material

The raw whole-genome resequencing data have been deposited in the NCBI Short Read Archive (SRA) under accession numbers: SRR10687319–SRR10687346, SRR10687348–SRR10687383, SRR10687385–SRR10687397, SRR10687399–SRR10687403, SRR10687405–SRR10687410, SRR10687412–SRR10687417, SRR10687419, SRR10687421–SRR10687423, SRR10687425, SRR10687427–SRR10687433, SRR10687436–SRR10687445, SRR10687447–SRR10687464, SRR10687466–SRR10687473, SRR10687475, SRR10687478, SRR10687483–SRR10687486, SRR10687488–SRR10687510, SRR10687513–SRR10687517, SRR10687519–SRR10687528, SRR10687530, SRR10687532–SRR10687535, SRR10687537–SRR10687554.

Authors' contributions

Guangliang Gao, Qigui Wang and Xianzhi Zhao provided funds, designed and supervised the projects. Guangliang Gao, Xianzhi Zhao, Jing Li and Youhui Xie collected the samples and the phenotypic data. Guangliang Gao, Dengfeng Gao; Silu Hu; Songsong Xu and Rui Wu, performed the bioinformatics analysis, data uploaded and drew the figures. Guangliang Gao, Keshan Zhang, Rui Wu and Chunhui Yin wrote the manuscripts.

Ethics approval and consent to participate

All experiments involving animals were performed according to the laws and regulations established by the Ministry of Agriculture of China (Beijing, China). The study was approved by the Animal Care and Welfare Committee of the Chinese Chongqing Academy of Animal Science (Chongqing, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1
The statistic of the BBW, EN48, EN60 and EYC in Sichuan White goose

Traits	Number	Mean	Stand dviation	Minimum	Maximum	CV (%)
BBW, g	160	88.89	9.07	66	109	10.20%
EN48	207	35.37	10.76	8.00	58.00	30.42
EN60	208	61.16	17.57	16.00	95.00	28.73
EYC	209	5.60	1.52	2.00	18.09	27.14

Table 2
Summary of the 26 SNP markers BBW, EN48, EN60 and EYC s in goose

SNP	Chromosome	Position (bp)	P value	Traits	Location/Nearest gene	Gene description
SNP222	chr33	1516391	1.01 × 10 ⁻⁰⁷	BBW	SMAP2	Stromal membrane-associated protein 2
SNP284	chr13	20688708	6.79 × 10 ⁻⁰⁸	EN48	PGM2L	Glucose 1,6-bisphosphate synthase
SNP283	chr3	49267770	4.03 × 10 ⁻⁰⁸	EN48	DP13B	DCC-interacting protein 13-beta
SNP285	chr3	49260834	7.62 × 10 ⁻⁰⁸	EN48	DP13B	DCC-interacting protein 13-beta
SNP286	chr3	38384739	8.20 × 10 ⁻⁰⁸	EN48	RTJK	RNA-directed DNA polymerase from mobile element jockey
SNP281	chr35	4541709	1.64 × 10 ⁻⁰⁸	EN48	TMEM161A	Transmembrane protein 161A
SNP282	chr35	4530609	3.59 × 10 ⁻⁰⁸	EN48	TMEM161A	Transmembrane protein 161A
SNP18	chr35	4512855	1.08 × 10 ⁻⁰⁷	EN60	S2542	Mitochondrial coenzyme A transporter SLC25A42
SNP287	chr35	4525296	8.29 × 10 ⁻⁰⁹	EN60	TMEM161A	Transmembrane protein 161A

SNP	Chromosome	Position (bp)	<i>P</i> value	Traits	Location/Nearest gene	Gene description
SNP288	chr35	4525312	8.29 × 10 ⁻⁰⁹	EN60	TMEM161A	Transmembrane protein 161A
SNP198	chr26	443100	9.69 × 10 ⁻⁰⁸	EYC	ZBT46	Zinc finger and BTB domain-containing protein 46
SNP188	chr30	3532845	8.33 × 10 ⁻⁰⁹	EYC	MBB1A	Myb-binding protein 1A-like protein
SNP189	chr30	3536078	1.45 × 10 ⁻⁰⁸	EYC	MBB1A	Myb-binding protein 1A-like protein
SNP190	chr30	3536533	1.45 × 10 ⁻⁰⁸	EYC	MBB1A	Myb-binding protein 1A-like protein
SNP191	chr36	1514622	2.44 × 10 ⁻⁰⁸	EYC	CB042	Uncharacterized protein C2orf42
SNP192	chr36	1514623	2.44 × 10 ⁻⁰⁸	EYC	CB042	Uncharacterized protein C2orf42
SNP196	chr36	1510734	5.65 × 10 ⁻⁰⁸	EYC	CB042	Uncharacterized protein C2orf42
SNP193	chr5	25366779	2.91 × 10 ⁻⁰⁸	EYC	CACB2	Voltage-dependent L-type calcium channel subunit beta-2
SNP194	chr5	25366791	2.91 × 10 ⁻⁰⁸	EYC	CACB2	Voltage-dependent L-type calcium channel subunit beta-2

SNP	Chromosome	Position (bp)	<i>P</i> value	Traits	Location/Nearest gene	Gene description
SNP183	chr5	21235021	2.95 × 10 ⁻¹³	EYC	CALCR	Calcitonin receptor
SNP184	chr5	21235070	9.24 × 10 ⁻¹¹	EYC	CALCR	Calcitonin receptor
SNP186	chr5	21249496	7.45 × 10 ⁻¹⁰	EYC	CALCR	Calcitonin receptor
SNP187	chr5	21363580	6.31 × 10 ⁻⁰⁹	EYC	CALCR	Calcitonin receptor
SNP185	chr5	21232365	2.43 × 10 ⁻¹⁰	EYC	GLP1R	glucagon-like peptide 1 receptor
SNP197	chr5	21069009	6.13 × 10 ⁻⁰⁸	EYC	TFPI2	Tissue factor pathway inhibitor 2
SNP195	ctg2610	34314	3.30 × 10 ⁻⁰⁸	EYC	CCM2	Cerebral cavernous malformations protein 2 homolog

Table 3
Effects of the SNPs and genotypes on EYC, EN48 and EN60 traits in goose

Traits	Genotypes (least-squares means)			P value
EYC				
SNP183	6.86 ± 0.31 ^a (AG)	5.45 ± 0.11 ^a (AG)	/	< 0.0001 ^{**}
SNP184	6.86 ± 0.31 ^a (CT)	5.43 ± 0.11 ^b (TT)	/	0.0001 ^{**}
SNP185	6.86 ± 0.31 ^a (GA)	5.45 ± 0.11 ^b (GG)	/	< 0.0001 ^{**}
SNP187	5.44 ± 0.11 ^b (GG)	6.68 ± 0.29 ^a (AG)	/	< 0.0001 ^{**}
SNP197	5.42 ± 0.11 ^b (GT)	6.66 ± 0.27 ^a (GG)	/	< 0.0001 ^{**}
EN48				
SNP17	36.86 ± 0.23 ^a (CC)	30.87 ± 1.67 ^b (TC)	26.71 ± 3.95 ^c (TT)	0.0019 ^{**}
SNP18	36.56 ± 0.85 ^a (AA)	33.85 ± 1.81 ^b (AG)	28.42 ± 2.42 ^c (GG)	0.0049 ^{**}
SNP281	37.57 ± 0.78 ^a (AA)	36.00 ± 0.99 ^{ab} (AG)	27.00 ± 1.51 ^b (GG)	< 0.0001 ^{**}
SNP282	28.25 ± 0.59 ^b (AA)	27.85 ± 1.59 ^b (AT)	37.45 ± 0.79 ^a (TT)	< 0.0001 ^{**}
EN60				
SNP17	64.55 ± 1.30 ^a (CC)	50.78 ± 2.61 ^b (TC)	43.57 ± 6.24 ^c (TT)	< 0.0001 ^{**}
SNP18	64.49 ± 1.34 ^a (AA)	54.26 ± 2.85 ^b (AG)	47.25 ± 3.72 ^c (GG)	< 0.0001 ^{**}
SNP281	66.00 ± 1.67 ^a (AA)	49.67 ± 2.54 ^b (AG)	64.14 ± 1.30 ^a (GG)	< 0.0001 ^{**}
SNP282	53.75 ± 0.84 ^b (AA)	50.15 ± 2.65 ^b (AT)	64.06 ± 1.31 ^a (TT)	< 0.0001 ^{**}
Note: *significant association P < 0.05; ** extremely significant association P < 0.01; Different letters in the same row indicate a significant difference between the genotypes (P < 0.05); the same letter indicates no significant difference between genotypes (P > 0.05).				

Figures

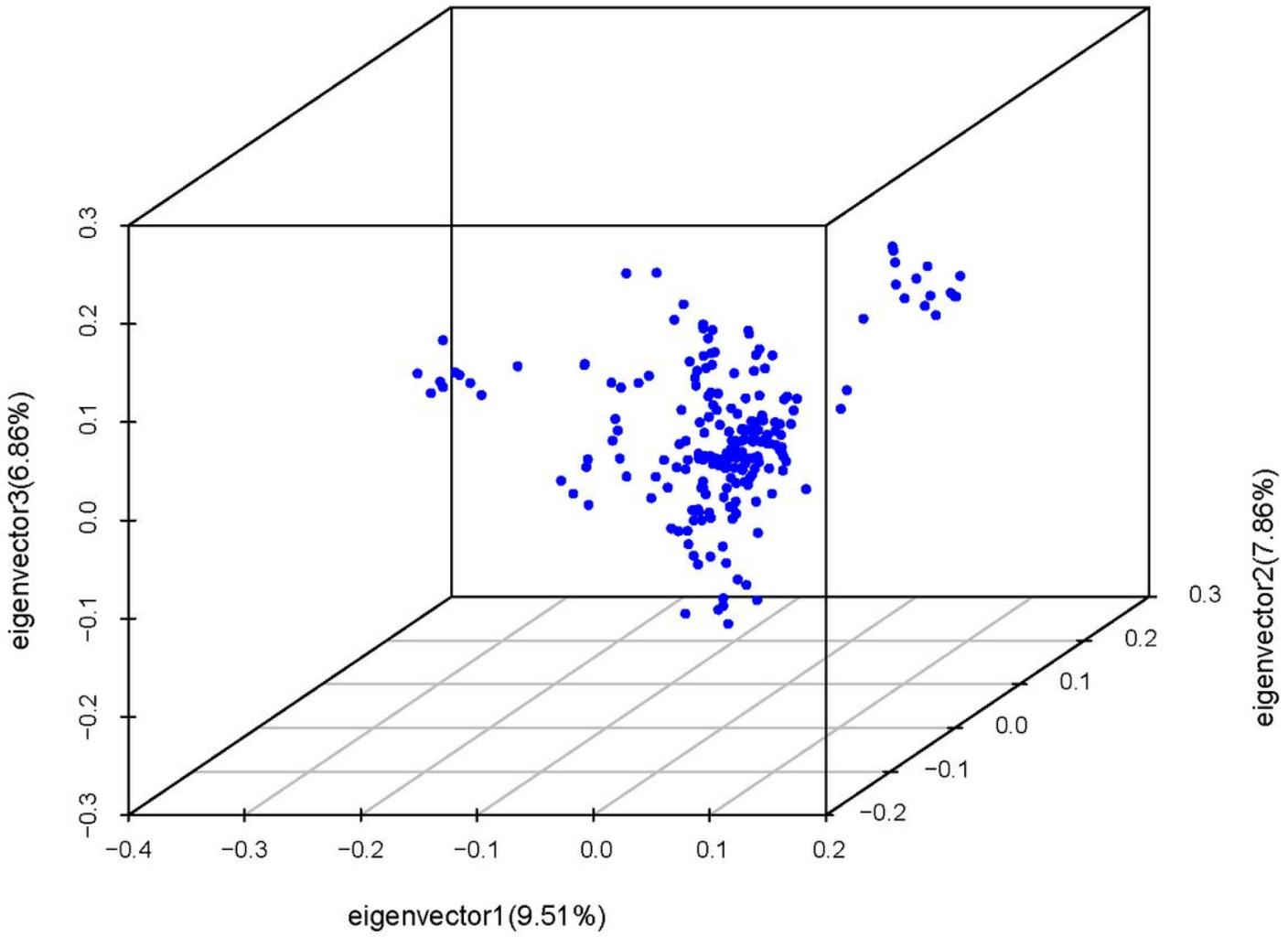


Figure 1

Figure 1

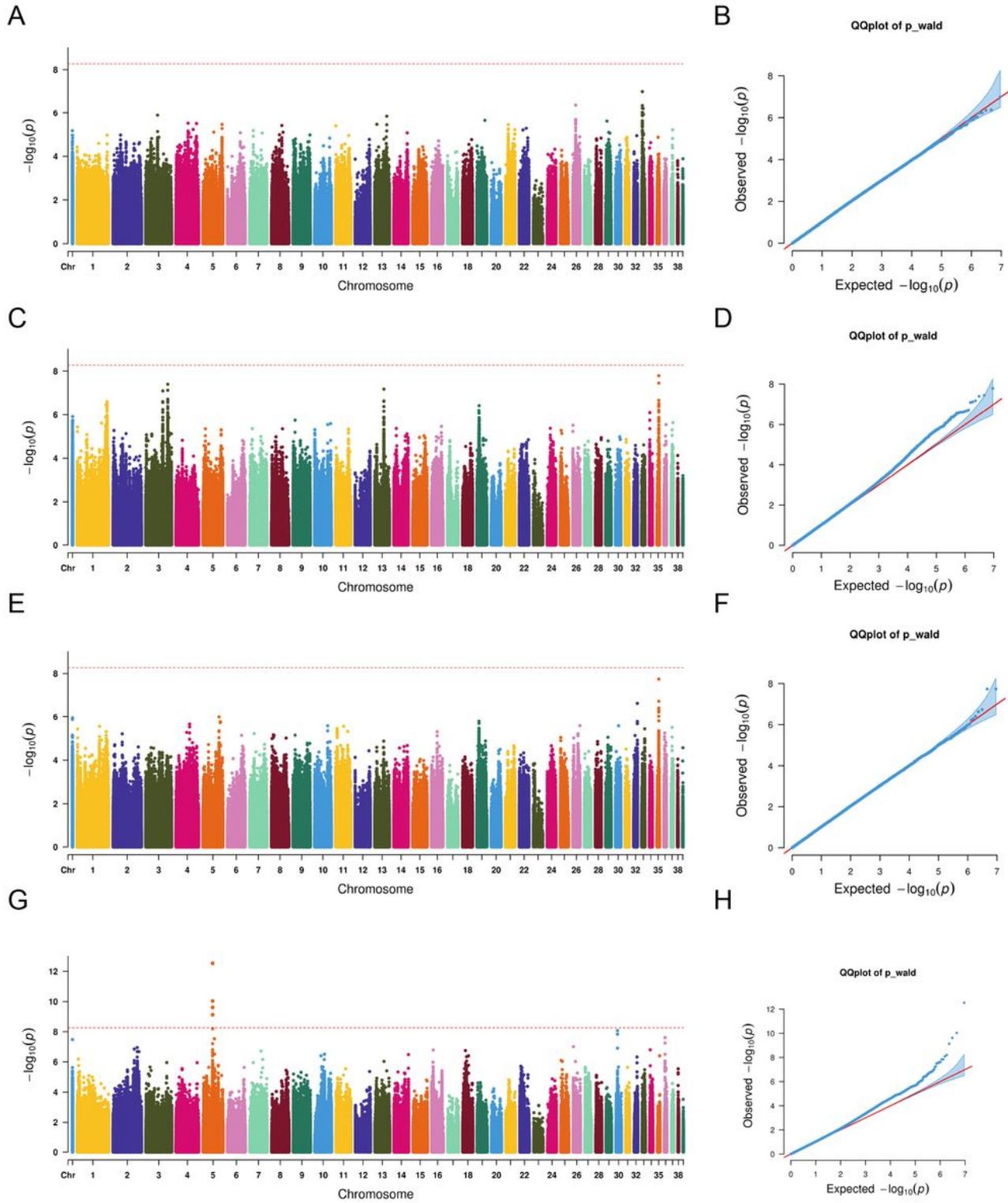


Figure 2

Figure 2

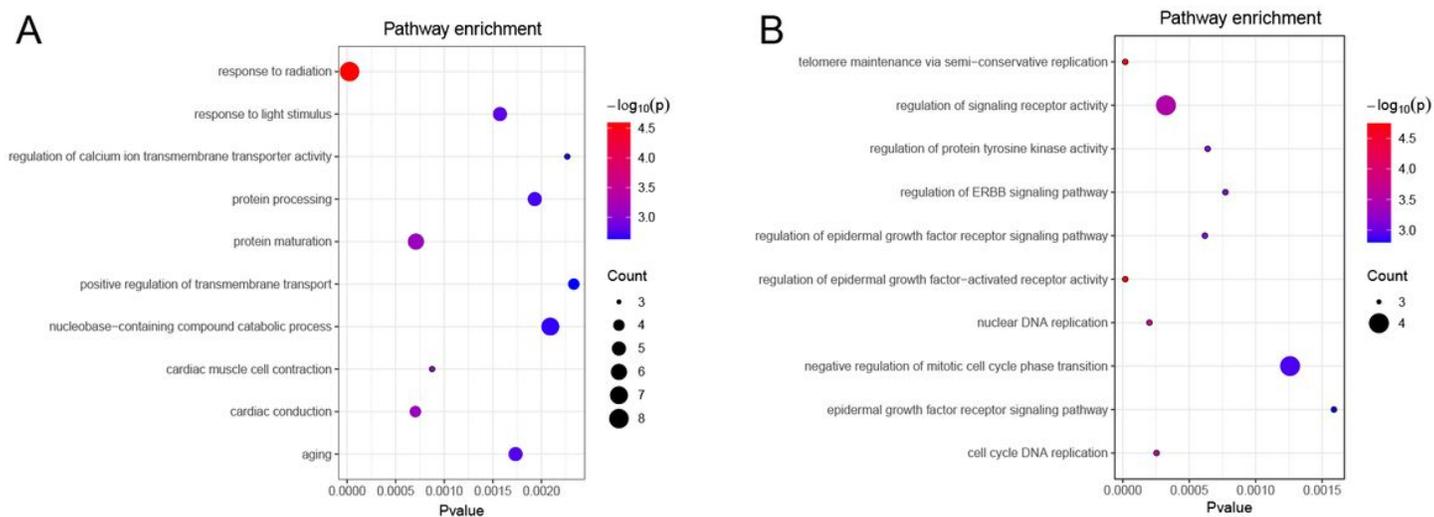


Figure 3

Figure 3

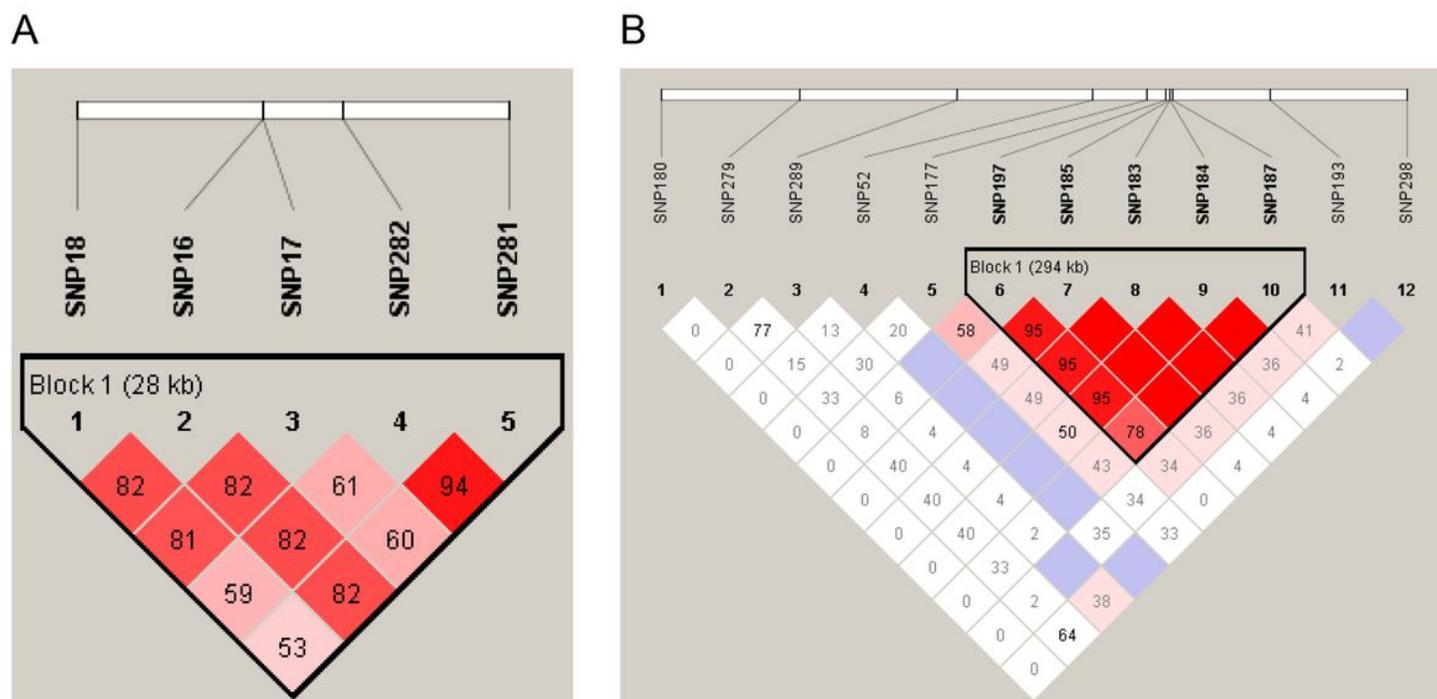


Figure 4

Figure 4

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