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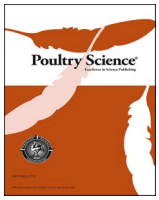

Identification of quantitative trait loci and candidate genes associated with growth curve parameters in chinese wenshang barred chickens☆

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ABSTRACT

The growth curve is a vital instrument for assessing and forecasting weight and developmental shifts in livestock and poultry, which reflects the changes of bodyweight traits with time and plays a key role in guiding breeding and production approaches. This study performed a genome-wide association study (GWAS) for growth curve parameters generated by nonlinear models which fit original weight-age records, to discover the SNPs and candidate genes correlated with growth traits. Data from 362 Chinese Wenshang Barred Chickens weighed at the age of 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18 weeks were used to fit the Gompertz, Logistic, and von Bertalanffy growth models. The Gompertz model showed the highest coefficient of determination ($R^2 = 0.974$). The mature body weight (**A**), time scale (**b**), and maturity rate (**k**) were treated as phenotypes for single-trait GWAS. The GWAS identified 44, 103, and 5 significant single nucleotide polymorphisms (**SNPs**) associated with A, b, and K, respectively. Among them, several candidate genes, including *LDB2, TOB2, RCBTB1, KPNA3, SLIT2, LCORL, LAP3*, and *TPRA1*, were previously reported to be associated with growth and development. Two lead SNPs (4:76022389, 4:76070237) on the *LDB2* gene were significantly associated with the growth curve. Further research of these candidate genes could help explore the full genetic architecture underlying growth and development traits in poultry.

Introduction

The growth curve, typically exhibiting an S-shaped pattern, visually represents the progressive changes in an animal's weight or development with age (Forni et al., 2009). It is a fundamental tool in analyzing growth and developmental patterns in livestock and poultry, underpinning breeding and production strategies. A sophisticated growth curve model enhances our understanding of the growth dynamics in these animals, enabling the prediction of developmental trends and facilitates comparative assessments of genetic traits across breeds and sexes. This information is instrumental in guiding breeding management. Growth traits are assessed by body weight at specific ages. Identified quantitative trait locus (**QTLs**) and genes linked to growth traits do not fully explain the observed growth pattern. Since animal growth is nonlinear, it is best understood through non-linear functions (Forni et al., 2009). These functions simplify weight-age data into a few key parameters, such as mature weight and maturity rate, which represent the individual animal's growth during rearing (Aggrey, 2002; Ghaderi-Zefrehei et al., 2024; Nguyen Hoang et al., 2021).

Several studies have reported the results of genome-wide association studies (**GWASs**) analyzing body weight measurements at various ages in chickens, including IGFs (Rubin et al., 2010; Wang et al., 2017) and *GHSR* (Fang et al., 2010). Furthermore, *IGF2BP1* has been shown to correlate positively with breast muscle weight and body size in various animals (Sutter et al., 2007; Wang et al., 2021; Zhou et al., 2018). The *LDB2* gene was responsible for BW at 7 to 12 weeks and weight gain at 6 to 12 weeks (Gu et al., 2011). The chicken QTL database (release 45) includes 4,776 QTLs related to growth traits, including BW at various

☆ Identification of genes for growth traits

The genome sequence data reported in this article is being uploaded to the genome sequence file of the BIG Data Center of the Beijing Institute of Genomics, Chinese Academy of Sciences, and is publicly available from http://bigd.big.ac.cn (CRA006685).

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ages and average daily gain (Hu et al., 2019). However, many QTLs, particularly those identified in previous studies, focus on a single data record, such as birth weight, weight before slaughter, and body weight measurements at specific ages. Detected QTL and genes associated with growth traits cannot clarify the growth pattern (Seifi Moroudi et al., 2021).

It is universally acknowledged that native animals are a precious asset and a vital resource for local areas. Protecting and nurturing them is extremely valuable and significant (Hartmann et al., 2003). The genetic enhancement of indigenous poultry populations is a critical element in the foundational breeding strategies. The genetic assets of these indigenous chickens are vital for the development of breeds that are optimally suited for production and resilient to local environmental conditions (Hoffmann et al., 2004). The Chinese Wenshang Barred Chickens (eggs with light brown eggshell) is a slow growing breed and an important source of both meat and eggs. As an agro-product geographical indications of P.R. CHINA, Wenshang Barred Chickens is very popular among consumers. However, the lack of efficient breeding systems in the purebred lines has resulted in the inefficient application of additive effect of production traits. The ability to model a growth curve utilizing three or four non-linear functional parameters can significantly enhance the precision in explaining the growth phenomenon. This approach facilitates the comparison of an animal's development rate, optimization of management and feeding strategies, and provides guidance for strategic planning in animal production.

Although growth curve parameters are crucial for improving animal production management and efficiency, GWASs focusing on these characteristics was lacking. Few studies have explored the association between single nucleotide polymorphisms (**SNPs**) and their growth curves parameters (Seifi Moroudi et al., 2021; Wang et al., 2024b). Therefore, body weight measurements were used to extract three growth curve characteristics: mature body weight (**A**), time-scale parameter (**b**), and maturity rate (**k**). In this study, to better understand the genetic basis of chicken growth curve parameters, we carried out an association study to identify SNPs related to the growth curve parameters.

Materials and methods

Resource population and phenotypes collection

All handling and experimental procedures concerning the chickens used in this study followed the guidelines established by the Ministry of Science and Technology (Beijing, China). The Science Research Department of the Shandong Academy of Agricultural Sciences (SAAS; Jinan, China) approved this study (reference number 2021001).

This study used 362 Chinese Wenshang Barred (**WB**) chickens obtained from Jinqiu Agriculture and Animal Husbandry Co., Ltd. (Wenshang, Shandong, China). The body weights (**BW**) were measured at 0–18 weeks and recorded biweekly. All the phenotypic data were distributed within the mean ± 3 standard deviations range and passed quality control for subsequent growth curve fitting (Table 1 and Fig. S1).

Table 1
Descriptive statistics of body weight in Chinese WB chickens.

Weeks (w)	N	Mean	SD	Min	Max	CV.
0	362	34.12	2.74	24	40	8.03 %
2	362	107.79	9.58	80	131	8.89 %
4	362	234.49	22.08	176	284	9.42 %
6	362	404.71	36.37	307	496	8.99 %
8	362	555.64	51.25	436	682	9.22 %
10	362	712.97	68.58	529	923	9.62 %
12	362	896.48	78.87	692	1125	8.80 %
14	362	1039.96	87.5	821	1284	8.41 %
16	362	1144.05	97.39	905	1415	8.51 %
18	362	1262.59	112.45	962	1577	8.91 %

N Number of animals, Mean Mean value, SD Standard deviation, Max Maximum, Min Minimum, CV. Coefficient of variation.

Genotyping and quality control

We collected blood samples from the wing vein (Table S1) and extracted genomic DNA using the phenol-chloroform method. The DNA quality was assessed by agarose gel electrophoresis, and paired-end (2 × 150 bp) DNA libraries were constructed for each sample. The DNBSEQ sequencing platform (BGI Genomics, Shenzhen, China) was used to obtain sequence data for all libraries.

Sequencing raw data was filtered with SOAPnuke (v1.5.6) (Chen et al., 2018). The specific parameters were filter -n 0.1 -l 20 -q 0.5 -Q 2 -G (https://github.com/BGI-flexlab/SOAPnuke). The Burrows-Wheeler aligner software (Li and Durbin, 2010) was used to align clean data to the chicken reference genome (http://ftp.ensembl.org/pub/release-106/fasta/gallus_gallus/), and the Samtools software (Li et al., 2009) was used to sort the aligned sequences according to the coordinates on the genome. The Qualimap 2 tool (Okonechnikov et al., 2016) was used to obtain summary statistics to assess the effectiveness of read mapping and alignment quality. The Samtools software (Li et al., 2009) filtered out the reads with quality values under 30. SNPs were called using GATK HaplotypeCaller v3.3 (McKenna et al., 2010) basing the SNP filtering conditions on the following: quality by depth < 2.0, Fisher strand > 60.0, root mean square of mapping quality < 40.0, MQRankSum < –12.5, HaplotypeScore > 13.0, and ReadPosRankSum < –8.0. SNPs were deleted based on minor allele frequency < 0.05 and SNP call rate < 0.05. The above was realized by Plink software(Purcell et al., 2007), and the parameters were: – maf 0.05 – geno 0.05. Finally, 9,381, 101 SNPs on 28 autosomal chromosomes were generated for analysis.

Growth curve fitting

Three of the most widely used nonlinear models (Table 2) to describe animal growth curves (Gompertz, Logistic, and von Bertalanffy) were fitted for each animal using the nlme package in R. The mature body weight function (**A**) is the ultimate body weight of an individual; the time-scale parameter (**b**) is the time for an individual to reach its maximum growth rate; the maturity rate (**k**) is the rate at which an individual approaches its mature body weight (**A**) (Fig. S2).

Estimation of genetic parameters

SNP-based heritability (h^2 SNP) was calculated using the GCTA v1.93.2 beta software (Yang et al., 2011) based on the genetic relationship matrix (**GRM**) between pairs of individuals (Li et al., 2022). The restricted maximum likelihood method was used for genetic parameter estimation. The genetic-statistical model was defined as follows:

$$Y_i = X_i b_i + Z_i u_i + e_i$$

where Y_i is a vector of clutch traits; X_i and Z_i are incidence matrices for b_i and u_i, respectively; b_i is a fixed effect vector; u_i is a polygenic effects vector with a variance-covariance structure of $u \sim N(0, G\sigma_u^2)$ in which G is the GRM between individuals and σ_u^2 is the polygenic variance; e_i is a random residual effects vector with $e_i \sim N(0, I\sigma_e^2)$ in which I is an identity matrix of dimension $n \times n$ (where our sample was $n = 362$).

Genome-wide association study for growth curve parameters in Wenshang barred chicken

To investigate the genetic basis of the growth curve model, we analyzed the association between A, b, and K using the linear mixed model in the Genome-wide efficient mixed-model association (GEMMA) software (v0.98.4) (Lee et al., 2010) based on chickens genotyped by whole-genome sequencing. GWAS was performed as follows:

Table 2
Growth curve model.

Model	Equation	Inflection point weight	Inflection point week	Maximum weekly gain
Gompertz	$Y = A * e^{-Bexp(-Kt)}$	A/e	lnB/K	Kw
Logistic	$Y = A/(1 + B*e^{-Kt})$	$A/2$	lnB/K	$Kw/2$
von Bertalanffy	$Y = A(1 - B*e^{-Kt})^3$	$8A/27$	$ln3B/K$	$3Kw/2$

Growth curve model. A is the mature body weight, b is the time-scale parameter, K is the maturity rate, W is the observed body weight, t is the growth time, and e is the natural logarithm.

$$y = W\alpha + X\beta + u + e$$

where y denotes the vector of phenotypic values; W represents the vector of covariates, including a column of 1 s; α is the vector of the corresponding coefficients including the intercept; x represents the vector of marker genotypes; β denotes the effect size of the marker; u represents the vector of random polygenic effects; e is the vector of errors. Linkage disequilibrium (LD) pruning, conducted with a window size of 25 SNPs, a step of five SNPs, and an r^2 threshold of 0.2, yielded 837,332 independent SNP markers and LD blocks. Similarly, the Bonferroni test corrected the whole genome and suggestive significance thresholds (0.05/837,332 and 1/837,332, respectively). Manhattan and quantile-quantile (Q-Q) plots were visualized using the CMplot package in R.

Further analysis of QTL regions

Region-based association analysis is an efficient approach to identifying causal SNPs. Significant and adjacent SNPs associated with the analyzed traits were further investigated by region-based association analyses using the IntAssoPlot package in R (He et al., 2020). LD values for the regional association plots (r^2) were calculated using PLINK v1.9, and gene annotations were obtained from the Ensembl Project (GCA_000002315.5, retrieved in May 2024).

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corporation, Armonk, NY, USA) and GraphPad Prism 9.

Results

Growth curve fitting

The models are shown in Table 3, and the three plotted growth curves are shown in Fig. S3. The R^2 values for the Gompertz, Logistic, and von Bertalanffy models were 0.974, 0.971, and 0.974, respectively, suggesting that the Gompertz and von Bertalanffy model showed the best goodness of fit. Table 4 shows the weighted average, Gompertz model, Logistic model, and von Bertalanffy growth curves. The curves representing the Gompertz model and average body weight overlap almost completely, while some deviations are seen in the other curves. The Gompertz model indicated that the mature body weight parameter (A) of the Chinese WB chickens was 1574.5 grams, which falls within the typical mature weight range for this population. Therefore, the Gompertz model parameters were selected as phenotypes for the GWASs.

Table 4
Comparison between actual body weight and the weight fitted by the Gompertz, Logistic, and von Bertalanffy models.

Weeks (w)	Body weight (g)	Gompertz (g)	Logistic (g)	Von bertalanffy (g)
0	34.12	47.07	83.29	28.25
2	107.79	116.91	141.81	114.07
4	234.49	229.37	233.78	249.78
6	404.71	377.89	367.08	415.13
8	555.64	547.02	539.23	591.09
10	712.97	719.45	731.23	763.74
12	896.48	881.37	913.3	924.39
14	1039.96	1024.39	1061.3	1068.5
16	1144.05	1145.11	1167.1	1194.43
18	1262.59	1243.62	1236.2	1302.37

Body weight was the actual weight measured every two weeks, Gompertz was fit values based on the Gompertz model, Logistic was fit values based on the Logistic model, Von bertalanffy was fit values based on the Von bertalanffy model.

Data characteristics

The descriptions of the estimated main and derived growth curve parameters of the Gompertz model are shown in Table 5. The coefficients of variation of these parameters in the population ranged between 5.23 and 11.92 %. The SNP-based heritability estimates were medium for A and b (0.37±0.14 and 0.38±0.13, respectively) and high for K (0.48 ± 0.13).

Principal component analysis (PCA)

Fig. 1a shows the WB chicken population stratification based on the PCA. The population formed three distinct clusters, showing

Table 5
Statistical descriptions of the estimated main and derived growth curve parameters obtained by the Gompertz model.

Trait	N	Mean	SD	Max	Min	CV.	h^2
A	362	1590.85	189.66	2458.43	1136.05	11.92 %	0.37 ±0.14
b	362	3.54	0.19	4.14	3.08	5.23 %	0.38 ±0.13
k	362	0.15	0.02	0.2	0.1	10.54 %	0.48 ±0.13

A the mature body weight, b time scale, k maturity rate, N Number of animals, Mean Mean value, SD Standard deviation, Max Maximum, Min Minimum, CV. Coefficient of variation, h^2: heritability.

Table 3
The parameter estimated value of growth curve model for Chinese WB chickens.

Model	Parameter			R^2	Inflection point week	Inflection point weight	Maximum weekly gain
	A	b	k				
Gompertz	1574.47	3.51	0.15	0.974	8.37	579.22	86.88
Logistic	1336.76	15.05	0.29	0.971	9.35	668.38	96.92
Von Bertalanffy	1807.83	0.75	0.11	0.974	7.37	535.65	88.38

Growth curve model. A is the mature body weight, b is the time-scale parameter, K is the maturity rate.

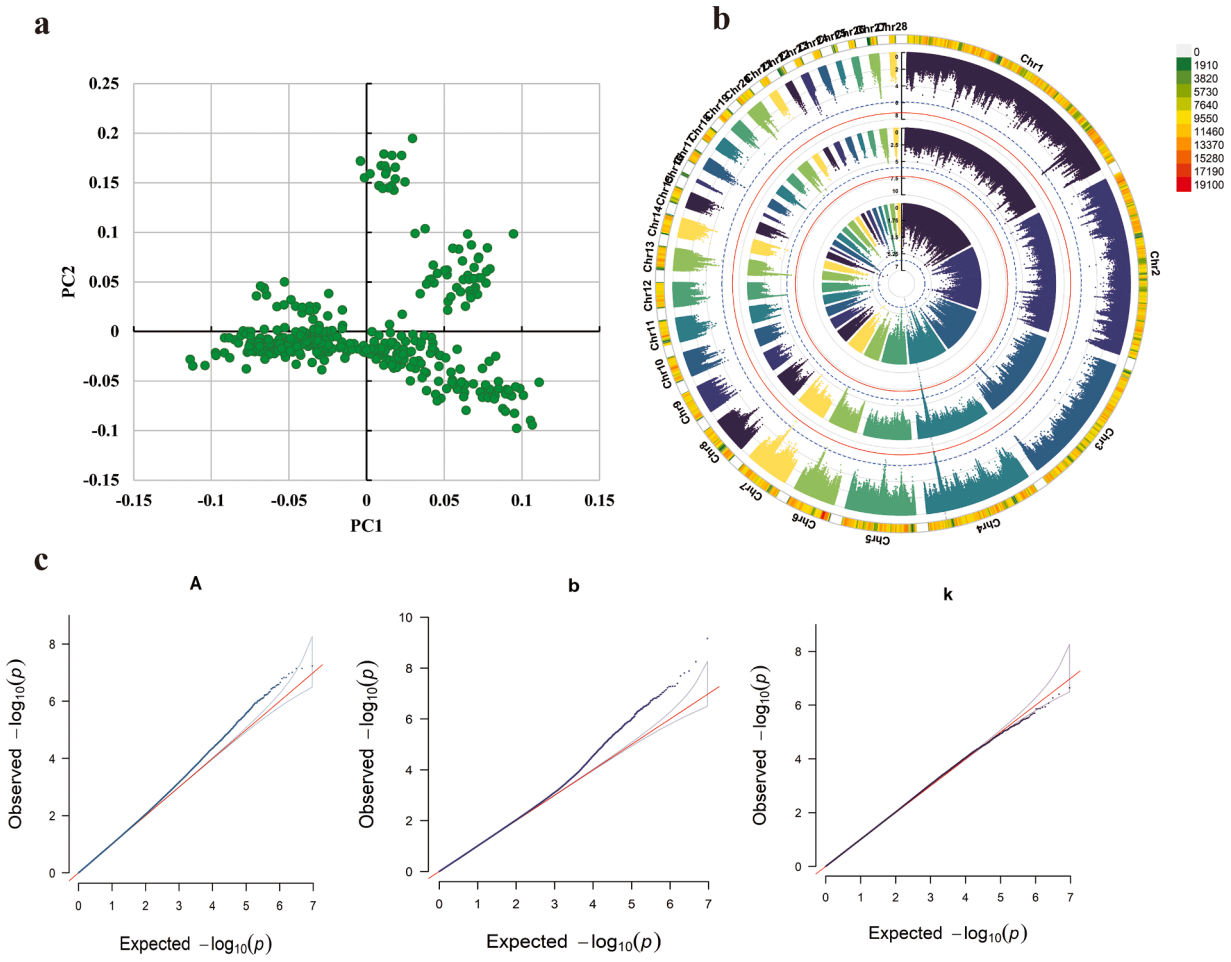

Fig. 1. Population genetic diversity and GWAS results. (a) PCA plot of the WB chicken breed. (b) A circle-Manhattan plot based on the GWAS for growth curve parameters. Each dot on this figure corresponds to a SNP in the dataset, and the horizontal red and blue lines denote the genome-wide significance (0.05/837,332) and the suggestive significance (1/837,332) thresholds, respectively. The Manhattan plot plots the –log10 observed *P*-values for genome-wide SNPs (Y-axis) against their corresponding position on each chromosome (X-axis). The horizontal axis represents the chromosome length in Mb. The colors correspond to SNP density. The circles from outside to inside are for the A, b, and K traits. (c) The Q-Q plots were derived from the GWASs for growth curve parameters. They plot the expected –log10-transformed *P*-values against the observed –log10-transformed *P*-values.

stratification within the reference group. Most individuals were clustered at the bottom center, with only a few scattered elsewhere. To eliminate the impact of this stratification on correlation analysis, the first three principal components were used as covariates.

GWAS analysis and gene function annotation

The GWAS Manhattan and Q-Q plots are shown in Fig. 1b and 1c. Most points were near the diagonal line in the Q-Q plots because the population structure was considered in the GWAS function, suggesting that this research had no inflation or systematic bias. The Manhattan plots detected 44 significant SNPs for mature body weight (**A**) on chromosomes (GGA) 1, 4, 5, 6, and 7, 103 SNPs for time-scale parameter (**b**) on GGA 1, 3, 4, and 13, and 5 SNPs for the maturity rate (**k**) on GGA 12, 15, 17, and 26. Several genes were identified as being involved in growth and development, including *KCNIP4, SLIT2, LCORL, LAP3, QDPR*, and *LDB2*. The detailed results are shown in Table 6, Table S2 and S3. In our previous study, we identified a region, located on chromosome 4 (7.41-7.64 Mb), was linked to body weight after ten weeks and body size traits. *LCORL, LDB2*, and *PPARGC1A* were identified as candidate genes (Wang et al., 2024a).

Table 6
The significant SNPs for the main growth curve parameters and their surrounding genes.

Trait	chr	Base-pair region		nSNP	Related genes
		Start	End		
A	1	49704023	-	1	*ZC3H7B / TOB2*
A	1	170043729	171069261	5	*ITM2B / PHF11 / RCBTB1 / KPNA3*
A	4	74844373	76112887	32	*KCNIP4 / SLIT2 / LCORL / LAP3 / QDPR / LDB2*
A	5	28731093	29522248	2	*RAD51B / GPHN*
A	6	25629986	-	1	*SORCS3*
A	7	23428996	24207945	3	-
b	1	170765139	-	1	-
b	3	88430605	88435695	5	*GSTA2 / TMEM14A*
b	4	74795317	76244653	91	*KCNIP4 / LCORL / LAP3 / LDB2*
b	13	4193239	4209684	6	-
k	12	10156584	-	1	*TPRA1*
k	15	7894924	7911713	2	-
k	17	3030231	-	1	*TNC*
k	26	2096412	-	1	*KLHDC8A*

A the mature body weight, b time scale, k maturity rate, Chr Chromosome, Base-pair region Physical position, nSNP number of SNPs in Base-pair region.

Region-based association test, linkage disequilibrium, and allele frequency analysis

The two region-based association plots for GGA 4, which included multiple SNPs in the LD-associated region, are shown in Figs. 2 and 3. The strongest associated SNP in one region on GGA 4 (74.84–76.11 Mb, 32 SNPs), located at 4:76022389 in the *LDB2* gene, was for mature body weight (A). LD analysis showed moderate LD levels for several haplotype blocks (Fig. 2a). We found that individuals of the AA type were larger than those of the TT type regardless of whether we assessed the growth curve parameters (A and b) or the growth curves (Fig. 2b, c, and Table 7). A leading SNP in another region on GGA 4 (74.79–76.24 Mb, 91 SNPs), located at 4:76070237 near the *LDB2* gene, was associated with the time-scale parameter (b). Individuals of the TT type had higher growth curve parameters (A and b) and growth curves than those with the CC type (Fig. 3b, c, and Table 7). Incorporation of the two SNPs at 4:76022389 and 4:76070237 into the model discovered no discernible superposition effect between them (Fig. S4).

Discussion

Non-linear models are often considered the best fit for modeling the growth of native poultry (González Ariza et al., 2021b; Mata-Estrada et al., 2020). The Gompertz, Logistic, and Richard's models are commonly used to depict the growth of native breeds. Their ability to fit growth patterns well is mainly due to their exponential functions (Darmani Kuhi et al., 2003). The Gompertz and von Bertalanffy models are commonly used to model the growth of local genetic types (González Ariza et al., 2021a). In this study, the R^2 of the Gompertz model was 0.999, the highest of the three models tested. The Gompertz model showed that the mature body weight parameter (**A**) of the Chinese WB chickens was 1574.5 g, within the normal mature weight range for the population. Although the R^2 of the Logistic and von Bertalanffy models were above 0.990, their mature body weight parameter A (1,336.8 and 1,807.8) was inconsistent with the actual weight of Chinese WB chicken. These results indicated that these two models might not be suitable for fitting the data assessed in this study. Therefore, the Gompertz model was chosen as the best model for Chinese WB chicken. Although there are few studies about growth curves in Chinese WB chickens, some authors have concluded that the Gompertz model provided the best fit for the body weight of other local chicken breeds (Aggrey, 2002; Rizzi et al., 2013; Soglia et al., 2020).

We performed GWAS for growth curve characteristics traits of Chinese WB chickens, identifying many genes involved in growth and development. These genes cannot be ignored since they influence most growth traits (Dekkers, 2004). For mature body weight (A), the 44 significant SNPs found on chromosomes 1, 4, 5, 6, 7, and 15 were associated with the *ZC3H7B, TOB2, ITM2B, PHF11, RCBTB1, KPNA3, KCNIP4, SLIT2, LCORL, LAP3, QDPR, LDB2, RAD51B, GPHN,* and *SORCS3* genes. For the time-scale parameter (b), the 96 significant SNPs found on chromosomes 1, 3, 4, and 6 were associated with the *GSTA2, TMEM14A, KCNIP4, LCORL, LAP3,* and *LDB2* genes. For the maturity rate (**K**), the five significant SNPs found on chromosomes 12, 15, 17, and 26 were associated with the *TPRA1, TNC,* and *KLHDC8A* genes. TOB2, a protein that stops cell growth, speeds up the removal of the poly(A) tail from mRNA by bridging between Caf1 deadenylase and the poly(A)-binding protein (**PABP**) plays a crucial role in regulating mRNA degradation. (Chen et al., 2020). When TOB2 is phosphorylated, it controls the overall rate at which mRNA is degraded, changing the mix of expressed genes and affecting how cells grow. TOB2 also slows down the formation of osteoclasts by lowering RANKL expression through its connection with vitamin D (3) receptor (Ajima et al., 2008). Researchers used chickens as a model bird species to create a detailed map of chromatin accessibility by analyzing 53 ATAC-seq samples from 11 different tissues. They pinpointed *RCBTB1* as a potential gene that controls body weight in chickens, particularly the weight of the muscle and liver

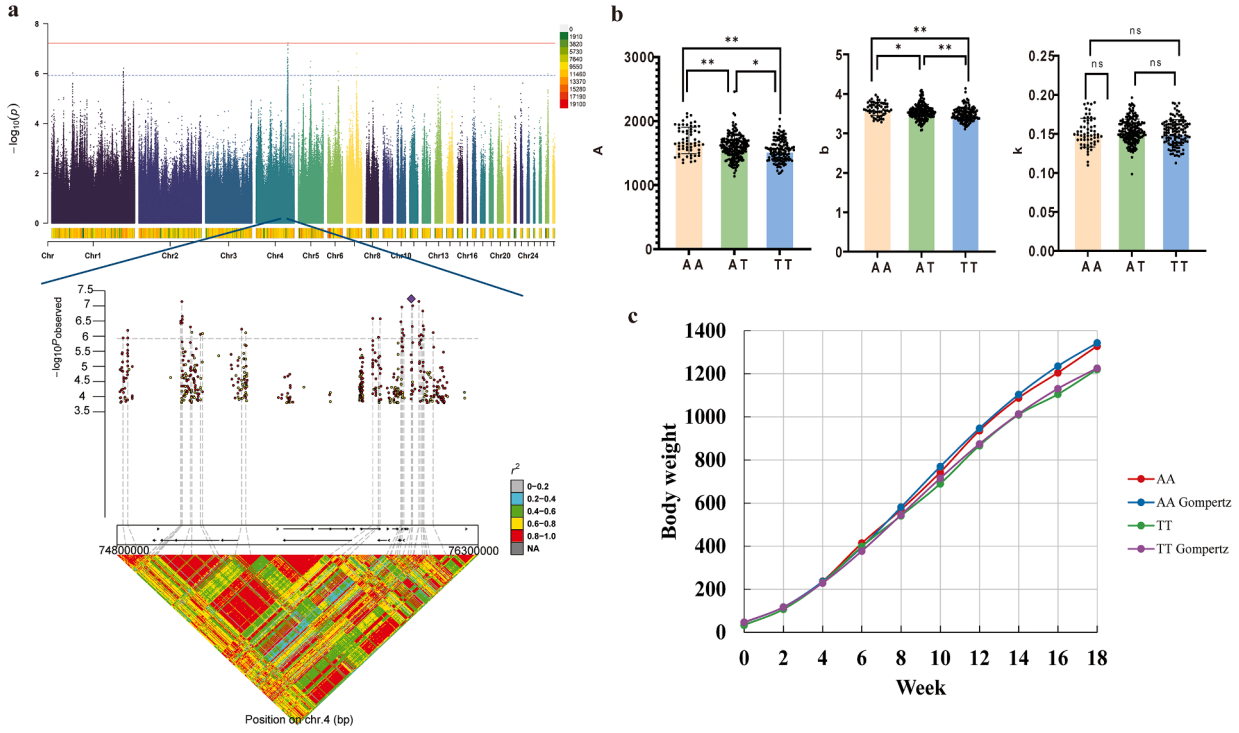

Fig. 2. Regional association analysis of the significant signal (GGA 4) with A. (a) A regional association plot and linkage disequilibrium (LD) blocks of the significant signal (GGA 4:74.84–76.11 Mb). (b) Phenotypic differences between individuals with different genotypes at 4:76022389 on GGA 4. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. (c) Growth curves of individuals with different genotypes at 4:76022389 on GGA 4.

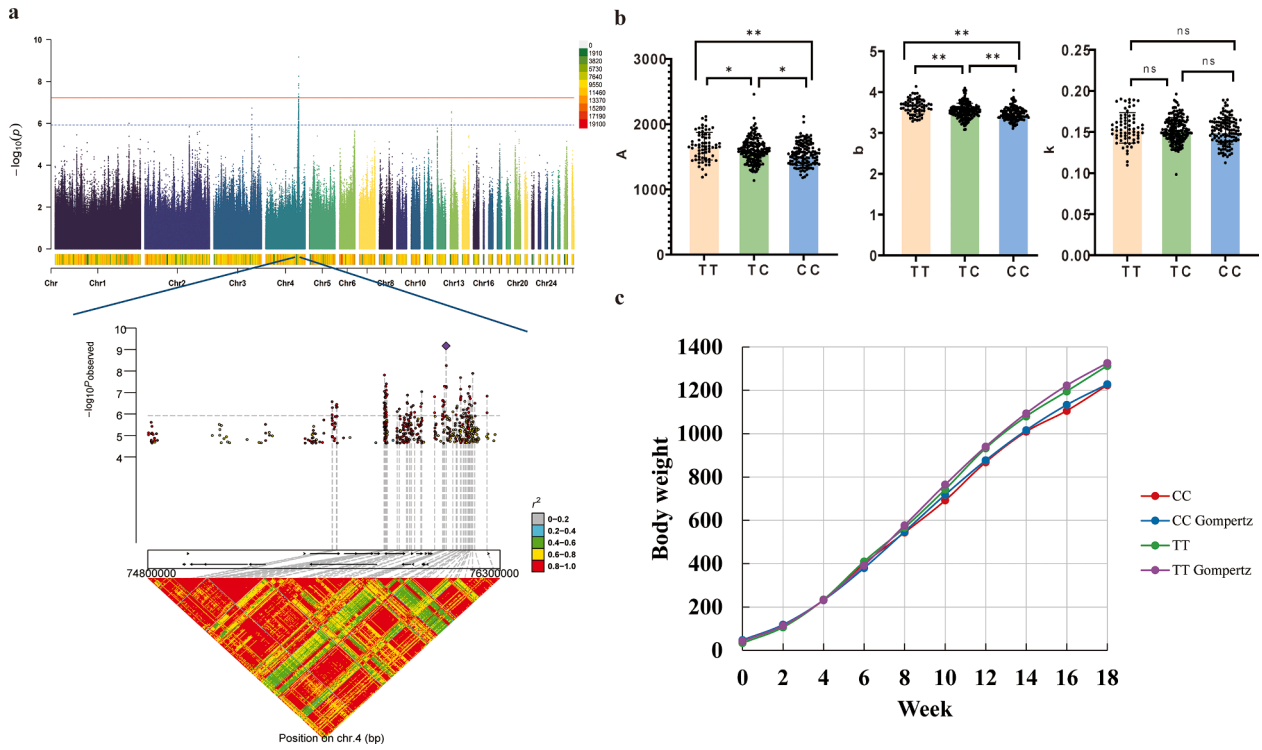

Fig. 3. Regional association analysis of the significant signal (GGA 4) with b. (a) A regional association plot and linkage disequilibrium (LD) blocks of the significant signal (GGA 4:74.79–76.24 Mb). (b) Phenotypic differences between individuals with different genotypes at 4:76070237 on GGA 4. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. (c) Growth curves of individuals with different genotypes at 4:76070237 on GGA 4.

Table 7
Comparison of significant SNPs of distinct genotypes corresponding to the weight (g).

Weeks (w)	4:76022389			4:76070237		
	AA($n = 59$)	AT($n = 183$)	TT($n = 120$)	CC($n = 123$)	CT($n = 172$)	TT($n = 67$)
0	35.06±2.73^{A}	34.04±2.94^{B}	33.80±2.32^{B}	33.80±2.43	34.21±2.84	34.48±2.98
2	107.97±10.51	107.92±9.58	107.50±9.18	108.07±8.60	107.98±9.45	106.79±11.52
4	237.69±23.96	235.23±21.57	231.79±21.78	233.05±20.68	235.47±21.62	234.63±25.65
6	414.31±40.60^{A}	405.78±34.69^{AB}	398.35±35.78^{B}	400.41±35.11	405.58±34.41	410.34±42.65
8	570.15±54.49^{A}	560.20±50.71^{A}	541.54±47.45^{B}	544.09±48.09^{B}	559.65±49.64^{A}	566.54±57.41^{A}
10	743.12±77.00^{A}	718.32±64.80^{B}	690.00±62.69^{C}	692.02±62.13^{C}	716.44±64.73^{B}	742.54±77.45^{A}
12	936.19±82.11^{A}	903.17±73.08^{B}	866.77±75.34^{C}	868.08±73.34^{C}	902.78±74.58^{B}	932.45±82.15^{A}
14	1087.88±88.77^{A}	1044.26±81.28^{B}	1009.83±84.83^{C}	1008.99±84.45^{C}	1046.80±82.34^{B}	1079.25±87.44^{A}
16	1204.92±98.28^{A}	1149.95±90.25^{B}	1105.13±90.51^{C}	1105.70±89.83^{C}	1151.57±91.97^{B}	1195.15±97.53^{A}
18	1328.32±112.92^{A}	1268.98±109.4^{B}	1220.52±99.26^{C}	1223.46±100.65^{C}	1271.06±108.99^{B}	1312.66±118.66^{A}

The majuscule letters in the shoulder label indicate significant differences ($P < 0.05$).

tissues (Zhu et al., 2023). A GWAS of a 1.5 Mb section (173.5–175.0 Mb) on chicken chromosome 1 (**GGA1**) found it to be closely linked to chicken growth. The *KPNA3-FOXO1A* region in this section had the most significant impact on growth traits (Xie et al., 2012). *KCNIP4* was shown to be associated with the body weight of chickens (Jin et al., 2015). *SLIT2* was found to be associated with body weight in chickens at the ages of 35 and 41 days (Pértille et al., 2015). *LDB2*, which is a transcriptional regulator (Johnsen et al., 2009) and was found to affect the growth traits of BW and average daily gain in chicken(Wang et al., 2019).A GWAS of F2 populations showed that the *LDB2* gene was associated with body weight at the age of 7–12 weeks and the average daily gain at the age of 6–12 weeks (Gu et al., 2011). Research has suggested that the *NCAPG-LCORL* gene locus affects human height. GWASs in cattle and horses and whole-genome analyses in pigs and dogs showed that this gene locus is linked to body length and weight (Takasuga, 2016). *LAP3* gene variation may underlie variations in growth rates among species. The *LAP3* gene might also be important for muscle growth in sheep (Ge et al., 2022). In poultry, the *LAP3* gene was associated with chicken growth traits (Liu et al., 2013). The *QDPR* gene is another important gene regulating growth (Girish et al., 2024). *TPRA1* controls early embryonic cell division and boosts the hedgehog signaling pathway (Aki et al., 2008; Singh et al., 2015). *TPRA1* might play a role in the early stages of cattle growth, which could affect their body weight (Zepeda-Batista et al., 2021). In this study, we could identify some new genes, significant SNPs related to growth curve parameters in WB chicken, but the reported biological markers could be confirmed by further complementary studies to find the exact causative genetic variation related to these traits in chicken.

We focused on two SNPs (4:76022389: $A > T$ and 4:76070237: T > C) located on the *LDB2* gene. Several studies have found that the *LDB2* gene is important in regulating retinal development and the cell cycle. A previous study discovered that a 31-bp indel in the *LDB2* gene could serve as a potential genetic marker for molecular breeding (Wei et al., 2020). In this study, these two SNPs were located in the intron of *LDB2* gene and may regulate the expression of the *LDB2* gene through cis-regulatory mechanisms, thereby affecting the functionality of the

LDB2 gene and further influencing body weight and growth rate. The SNP 4:76022389 significantly affects mature body weight (**A**) and the body weight after 6 w. Based on this marker, the growth rate and final body weight of genotype AA were significantly greater than those of genotype TT, which had a higher growth rate than those with the CC genotype. Analysis revealed that the 4:76022389 mutation was associated with the time-scale parameter (b) and body weight after 8 w. In the breeding progress for WB chickens or other breeds, in order to increase or control the growth rate of individuals, we can select individuals with specific genotypes in the early stages, reduce costs, and improve breeding efficiency. Therefore, *LDB2* could be used as a molecular marker to improve the growth performance of chickens.

Conclusion

We used three growth curve models to fit the body weight data of Chinese WB chickens. We then used the parameters of the Gompertz model, the one with the best-fitting effect, as the phenotypes for the GWAS, finding 149 significant SNPs. Utilizing the growth curve parameter as input for GWAS offers a distinct advantage over the traditional approach, which relies on raw phenotype data at a specific time point. This new method captures the dynamic changes in traits over time. Several candidate genes were significantly associated with growth and development traits, including *LDB2, TOB2, RCBTB1, KPNA3, SLIT2, LCORL, LAP3*, and *TPRA1*. Although no molecular biological tests have been conducted to verify the function of the candidate genes, the role of the associated genes and SNPs in growth and development was also discussed. Further research on these candidate genes could help explore the full genetic architecture underlying growth and development traits in chickens.

Section for the paper

Genetics and Molecular Biology

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.104767.

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