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ORIGINAL ARTICLE

Genetic parameters estimation and genome-wide association studies for internal organ traits in an F_2 chicken population

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Abstract

Chicken internal organs are indispensable parts of the body, but their genetic architectures have not been commonly understood. Herein, we estimated the genetic parameters for heart weight (HW), liver weight (LW), spleen weight (SpW), testis weight (TW), glandular stomach weight (GSW), muscular stomach weight (MSW) and identified single nucleotide polymorphisms (SNPs) and potential candidate genes associated with internal organ weights in an F_2 population constructed by crossing broiler cocks derived from Arbor Acres with high abdominal fat content and Baier layer dams (a Chinese native breed). The restricted maximum likelihood (REML) method was applied for genetic parameters estimation of internal organ weights using GCTA software. The results showed that heritabilities of internal organ traits ranged from 0.336 to 0.673 and most of the genetic and phenotypic correlations amongst internal organs weights were positive. A genome-wide association study (GWAS) was performed based on a mixed linear model (MLM) in GEMMA software. Genotypic data were produced from the whole genome re-sequenced (26 F_0 individuals were re-sequenced at 10 × coverage; 519 F_2 individuals were re-sequenced at 3 × coverage). A total of 7,890,258 SNPs remained to be analysed after quality control and genotype imputation. The GWAS results indicated that significant SNPs responsible for internal organ traits were scattered on the different chicken chromosomes 1–5, 8, 11, 14, 16, 18, 19 and 27. Amongst the annotated genes, fibronectin type III domain containing 3A (*FNDC3A*), *LOC101748122*, membrane palmitoylated protein 6 (*MPP6*), *LOC107049584* and KAT8 regulatory NSL complex subunit 1 (*KANSL1*) were the most promising candidates for internal organ traits. The findings will provide instrumental information for understanding the genetic basis of internal organ development.

KEYWORDS

chicken, genetic parameters estimation, GWAS, internal organs

1 | INTRODUCTION

In the past decade, poultry breeding programmes have focused on weight gain, which has led to metabolic disorders and unhealthy changes in the internal organ (Deeb & Lamont, 2002; Grupioni et al., 2015). The body weight of the chicken is the sum of fat mass, internal organ mass, muscle mass and skeleton mass (Gaya et al., 2006). Each of these components has its developmental process (Zhang et al., 2007). Internal organs are closely linked and coordinated in their structure and functions. The heart acts as the core of the blood circulatory system (Larson & Ormiston, 1972) and cardiac capacities may become a limiting factor for broiler development (Gaya et al., 2006); the liver is a defence organ and is regarded as a regulator of metabolism and blood circulation (Michalopoulos & Bhushan, 2021); the spleen is an organ that combines the innate and adaptive immune system (Scothorne, 1985); the size and weight of the testes can be as a predictor for semen production and are positively correlated with semen production (Sun et al., 2018); the glandular stomach is characterized by the secrete gastric juice and the muscular stomach is responsible for storing and grinding food (Matsuda et al., 2005). As by-products of the meat economic system, internal organs also have great economic value, for example, the chicken stomach has been proved to have potential medicinal value (Andrée et al., 2010). The detection of genomic regions and underlying mutations associated with internal organ weight traits may facilitate the selection of chickens with proportional development of internal organs and reduce susceptibility to metabolic disorders (Moreira et al., 2019).

As an integral part of the chicken body, the genetic development of internal organs is coordinated with other traits. Gaya et al. (2006) revealed that the feed conversion ratio exhibits positive genetic correlations with internal organ weights in the commercial broilers although the weights of internal organs are not direct targets for selection in chickens. The estimation of genetic parameters showed that there was also a close relationship between chicken internal organs. Dou et al. (2019) revealed that genetic correlation coefficients between internal organ weights (Heart weight, HW; Liver weight, LW; Glandular stomach weight, GSW; Muscular stomach weight, MSW) were moderate to high, varying from 0.503 to 0.711. These data suggest that it is necessary to figure out the genetic relationship amongst internal organ traits by using the estimation of the genetic parameters prior to considering them to be selection objectives in breeding programmes.

In the recent two decades, a number of the quantitative trait loci (QTL) and candidate genes that significantly affect chicken internal organ weights have been identified based on marker-QTL linkage analysis and genome-wide association study (GWAS). Previous studies showed that QTLs for HW and LW at 9 weeks were detected on Gallus gallus chromosome 1 (GGA1) and GGA4, respectively, by using marker-QTL linkage analysis in an F_2 population (Navarro et al., 2005). Nones et al. (2006) identified some QTLs associated with MSW, LW, HW and lung weight located on GGA1 by conducting a linkage map in an F_2 chicken population. HW and GSW at 6 weeks were mapped to GGA13 by using an association study between microsatellites marker and traits in a Brazilian F_2 chicken reference population (Boschiero et al., 2009). Zhang, Yu, et al. (2017) identified that *TCF21* on GGA21 might be important for testis growth and development in chickens by GWAS (Zhang, Na, et al., 2017) and epistasis analysis (Zhang, Yu, et al., 2017). Dou et al. (2019) performed a GWAS on an F_2 resource population with a 600 K array and identified five candidate genes (*SHH*, *NCAPG*, *WDFY2*, *GTF2F2* and *HTR2A*) with significant effects on internal organ weights. Moreira et al. (2019) identified 14 positional candidate genes (PCGs) associated with internal organ traits in an F_2 chicken population by GWAS and biological functions annotation.

Here, we performed the estimation of genetic parameters and a GWAS to dissect the genetic architecture of internal organ traits in an F_2 chicken population. This study provides novel insights into the genetic architecture underlying chicken internal organ growth and development and is of significance for applying genomics in practical chicken breeding.

2 | MATERIALS AND METHODS

2.1 | Experimental populations and phenotypic measurements

The population was an F_2 population constructed by crossing broiler cocks derived from Arbor Acres with high abdominal fat content and Baier layer dams (a Chinese native breed) (Leng et al., 2009). More details about this population have been reported in previous studies (Zhang et al., 2010, 2011). A total of 519 F_2 birds from 12 half-sib families were used in this study. All F_2 birds had free access to feed and water in the process of feeding and were provided commercial diets based on corn and soybeans in line with all NRC (1994) requirements. All birds were euthanized by intramuscular injection of pentobarbital (Sigma, St. Louis, MO, USA) (40 mg/kg) under deep anaesthesia and exsanguination from the jugular vein at the age of 12 weeks. Then their body weights (BW_{12}) were recorded. After slaughtering, HW, LW, SpW, TW, MSW and GSW were manually separated and weighed.

2.2 | 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. The restricted maximum likelihood (REML) method was applied for genetic parameter estimation. The genetic-statistical model was defined as follows:

$$\boldsymbol{Y_i} = \boldsymbol{X_i b_i} + \boldsymbol{Z_i u_i} + \boldsymbol{e_i}$$

where $\boldsymbol{Y_i}$ is a vector of internal organ traits; $\boldsymbol{X_i}$ and $\boldsymbol{Z_i}$ are incidence matrices for $\boldsymbol{b_i}$ and respectively. $\boldsymbol{b_i}$ is a vector of fixed effect including sex and BW_{12}. $\boldsymbol{u_i}$ is a vector of polygenic effects with a variance-covariance structure of $\boldsymbol{u} \sim N(0, \boldsymbol{G}\sigma^2_{\mu})$, $\boldsymbol{G}$ is the GRM between individuals (Yang et al., 2010), σ^2_{μ} is the polygenic variance; $\boldsymbol{e_i}$ is a vector of random residual effects with $\boldsymbol{e_i} \sim N(0, \boldsymbol{I}\sigma^2_e)$, $\boldsymbol{I}$ is an identity matrix of dimension $n \times n$ (with n, the sample size = 519).

For pairwise genetic correlation (r_g) analysis of internal organ traits, bivariate Genome-based Restricted Maximum Likelihood (GREML) analysis was performed in GCTA v1.93.2. The phenotypic correlation (r_p) of pairs of traits (x and y) was calculated from the bivariate genomic REML outputs using the following formula:

$$r_{pxy} = \frac{\sigma_{uxy} + \sigma_{exy}}{\sqrt{\left(\sigma^2_{ux} + \sigma^2_{ex}\right) \times \left(\sigma^2_{uy} + \sigma^2_{ey}\right)}}$$

where σ_{uxy} and σ_{exy} were the genetic covariance and residual covariance between x and y, respectively; σ^2_{ux} and σ^2_{ex}, as well as σ^2_{uy} and σ^2_{ey} were the genetic variance and residual variance of x and y respectively. The standard errors of the phenotypic correlation were obtained using the following procedure (Sharma, 1998):

$$SE\left(r_p\right) = \sqrt{\frac{\left(1 - r^2_p\right)}{(n-2)}}$$

where n is the number of pairs of samples, r_p is the phenotypic correlation coefficient.

2.3 | Genome sequencing and quality control

The reagent test kit was used to extract total genomic DNA from the blood of each bird. Genome sequencing of each individual was performed by using the Illumina HiSeq PE150 platform (26 F_0 individuals; 519 F_2 individuals). Library construction and sample indexing were done according to the standard protocol of Illumina. The F_0 individuals were re-sequenced with an averaged depth of 10×, and F_2 individuals were re-sequenced with an averaged depth of 3×. SNP calling was performed on a population scale as implemented in the package SAMtools after alignment (Li et al., 2009). We calculated genotype likelihoods from reads for each individual at each genomic location and the allele frequencies in the sample. Only high-quality SNPs (coverage depth ≥2, root mean square mapping quality ≥20, miss ≤0.3) were kept for subsequent analysis to exclude SNP calling errors caused by incorrect mapping. A total of 10,889,955 SNPs were left after filter from 15,868,916 raw SNPs. The missing genotypes in F_2 population were filled based on the sequencing results of F_0 generation. Ten-fold cross-validation is used to test the accuracy of genotype filling. Imputation was performed using BEAGLE 4.0 (Browning & Browning, 2009) with default parameters settings. A total of 7,890,258 SNPs were left after the imputed 10,889,955 SNPs were filtered by MAF ≥0.05 and miss ≤0.2 for the 519 individuals.

2.4 | Single-marker GWAS

Association analysis was conducted using the GEMMA (Genome-wide Efficient Mixed-model Association) software package (Zhou & Stephens, 2012). For the MLM (mixed linear model) analysis, the equation was as follow:

$$Y = Xb + Sa + Zu + e$$

In this equation, $\boldsymbol{Y}$ represents phenotype; $\boldsymbol{X}$ is the incidence matrix with gender as a fixed effect and BW_{12} as a covariate and $\boldsymbol{b}$ is the vector of corresponding coefficients including the intercept; $\boldsymbol{S}$ represents the vector of SNP genotype and $\boldsymbol{\alpha}$ is the corresponding effect of the marker; $\boldsymbol{Z}$ is the incidence matrix of the random additive genetic effects vector $\boldsymbol{u}$ that follows the multinormal distribution $N(0, \boldsymbol{G}\sigma^2_{\mu})$, in which $\boldsymbol{G}$ is the genomic relationship matrix based on IBS - identity by state, and $\sigma_{\mu}{}^2$ is the polygenetic additive variance. $\boldsymbol{e}$ represents random residual with a distribution of $N(0, \boldsymbol{I}\sigma^2_e)$. Particularly, principal component analysis (PCA) revealed no significant PCs in this population, suggesting that there is no population stratification. Thus, PCs were not included in the mixed model. The genome-wide significance threshold value is set as $p < 10^{-6}$ to control the false positive rate (Ma et al., 2018).

2.5 | Functional annotation of the candidate genes

SNP annotation was performed according to the GCF_000002315.6_GRCg6a reference genome using

ANNOVAR (Wang et al., 2010). Only the high-quality SNPs were annotated. SNPs were categorized in exon regions, intronic regions, splicing sites (within 2 bp of a splicing junction), upstream and downstream regions (within a 1 kb region upstream or downstream), and intergenic regions based on the genome annotation. We identified PCGs according to the physical location of each significant SNP on GGA.

3 | RESULTS

3.1 | Descriptive statistics and genetic parameters estimation

Descriptive statistics and genetic parameters estimation for internal organ traits were summarized in Tables 1 and 2 respectively. Heritabilities of internal organ traits varied from 0.336 to 0.673. There were positive genetic and phenotypic correlations amongst most internal organ weights. The genetic and phenotypic correlations between TW and LW, MSW, GSW and the genetic correlations between LW and HW were negative.

3.2 | GWAS

The statistical results of GWAS for internal organ weights were shown by the Manhattan plot (Figure 1). The information of significant SNPs and a list of candidate genes screened by GWAS for each trait were shown in Table 3. Most of the detected significant SNPs of GSW were distributed within the genomic region from 157.90 to 170.38 Mb on GGA1 and there was a significant SNP in 0.67 Mb on GGA33. For HW, there were significant SNPs in the 170,867,693 bp on GGA1 and 29,047,258 bp on GGA4 respectively. For LW, there were significant SNPs on chromosomes 2, 8, 14, 18 and 19 respectively. Only one significant SNP located in 179,901,984 bp on GGA1 was associated with MSW. Most of the detected significant SNPs for SpW were distributed within the three genomic regions including 170.39–171.60 Mb on GGA1, 6.38–6.72 Mb on GGA3, 2.48–2.66 Mb on GGA16. The remaining significant SNPs for SpW were scattered on different genomic regions on GGA2, GGA4, GGA5, GGA11 and GGA27. A total of 17 significant SNPs for TW were detected, eight were within the 96.57–97.87 Mb on GGA1, and nine within 20.47–23.46 Mb on GGA7.

TABLE 1 The descriptive statistics of internal organ traits (in grammes) of F_2 chickens

Traits	N	Mean	SD	Min	Max	CV (%)
HW	514	9.90	2.78	4.68	18.22	28.11
LW	515	38.41	7.54	23.00	59.00	19.62
SpW	495	3.49	1.03	1.64	6.45	29.48
TW	258	10.15	7.05	0.69	31.18	69.44
MSW	508	22.41	4.22	13.38	34.98	18.81
GSW	502	6.29	1.25	2.32	9.77	19.88

Abbreviations: CV, coefficient of variation; GSW, glandular stomach weight. N, number of animals; HW, heart weight; LW, liver weight; Max, maximum; Min, minimum; MSW, muscular stomach weight; SD, standard deviation; SpW, spleen weight; TW, testis.

3.3 | Positional candidate genes and overlap with previously reported QTL

Of all the PCGs associated with internal organ weights in chickens we revealed (Table 4), multiple genes including fibronectin type III domain containing 3A (*FNDC3A*), *LOC101748122,* membrane palmitoylated protein 6 (*MPP6*), *LOC107049584,* KAT8 regulatory NSL complex subunit 1(*KANSL1*) have previously been annotated in the Chicken QTL database–release 45. The genomic regions of these genes overlapped with known QTL mapped for internal organ traits. Zhou et al. (2006) identified the genomic regions on Chromosome 2 for SpW in an F_2 chicken population through genome-wide association scan (QTL # 1886, QTL# 12571); Park et al. (2006) detected one genomic region for SpW on chromosome 11 using a reciprocal intercross comprising F_2 birds (QTL # 2287); Zhang et al. (2012) mapped one genomic region on Chromosome 27 for SpW from a Beijing-You chickens from 50 families by using 60K SNP Illumina iSelect chicken array (QTL # 2287). Dou et al.(2019) reported that genomic regions on chromosome 1 for HW, SpW, and GSW was mapped in an F_2 resource population generated from Dongxiang Blue-Shelled and White Leghorn chickens using a high-density Affymetrix 600 K SNP array (QTL # 170493, QTL # 170497, QTL # 170491, QTL # 170495).

4 | DISCUSSION

For over half a century, the goal of chicken breeding programmes, especially broiler, has mainly focused on improving essential economic production traits, such as growth rate and feeding efficiency (Le Bihan-Duval et al., 2011). However, there are no parallel improvements in the skeleton and internal organs to support the

TABLE 2 Heritabilities, phenotypic and genetic correlations of six internal organ traits in F_2 chickens

	HW	LW	SpW	TW	MSW	GSW
HW	0.336 (0.080)	0.080 (0.044)	0.118 (0.045)	0.065 (0.063)	0.076 (0.045)	0.030 (0.045)
LW	−0.060 (0.198)	0.352 (0.087)	0.187 (0.044)	−0.414 (0.057)	0.119 (0.044)	0.101 (0.045)
SpW	0.265 (0.157)	0.289 (0.152)	0.673 (0.080)	0.193 (0.064)	0.119 (0.045)	0.163 (0.045)
TW	0.157 (0.241)	−0.319 (0.216)	0.264 (0.186)	0.384 (0.134)	−0.078 (0.064)	−0.146 (0.044)
MSW	0.181 (0.160)	0.351 (0.152)	0.087 (0.129)	−0.024 (0.196)	0.643 (0.076)	0.365 (0.042)
GSW	0.106 (0.201)	0.199 (0.191)	0.279 (0.155)	−0.387 (0.212)	0.662 (0.115)	0.378 (0.091)

Abbreviations: GSW, glandular stomach weight; HW, heart length; LW, liver weight; MSW, muscular stomach weight; SpW, spleen weight; TW, testis weight.

[a]Heritability of traits with standard errors (on diagonal).

[b]Phenotypic correlation coefficients with standard errors (above diagonal).

[c]Genetic correlation coefficients with standard errors (below diagonal).

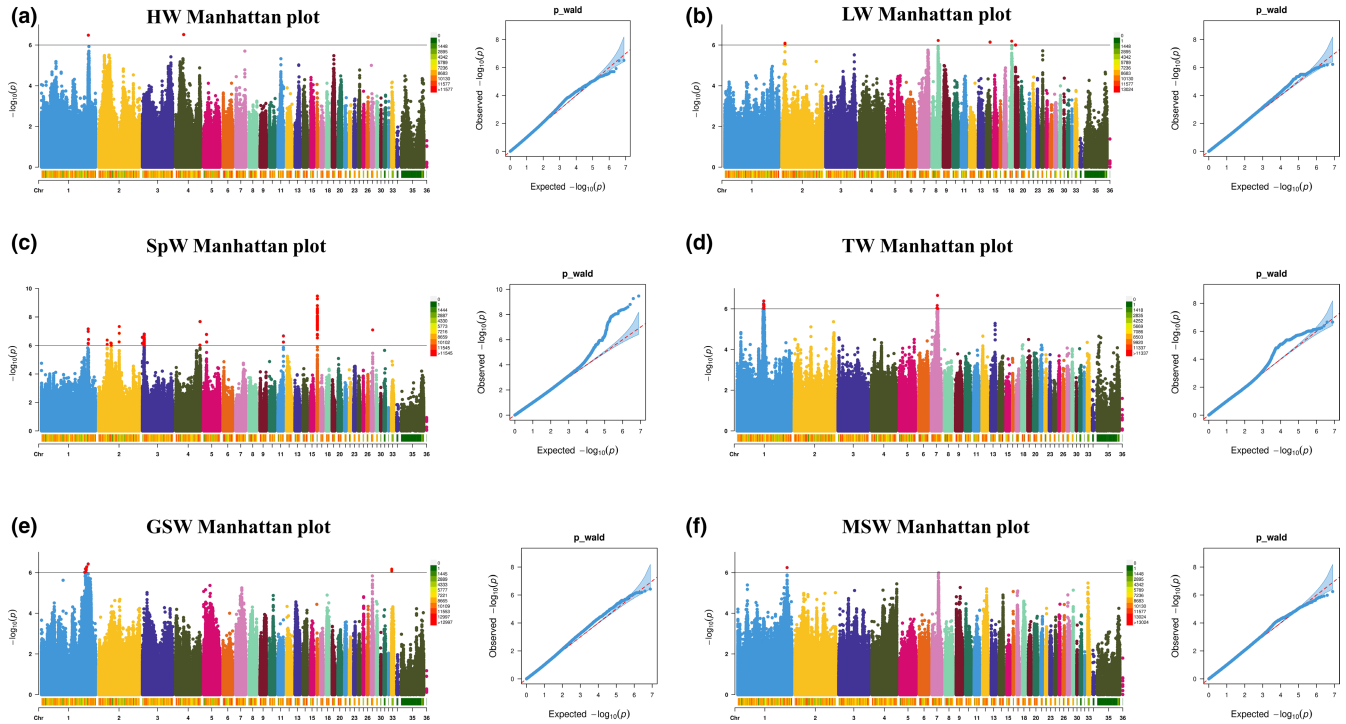

FIGURE 1 Manhattan and quantile-quantile (Q–Q) plot for the association analyses of 6 internal organ weight traits in the F_2 population. (a–f) Showed that the result of association analyses of the weights of heart (HW), liver (LW), spleen (SpW), testis (TW), glandular stomach (GSW) and muscular stomach (MSW). In the Manhattan plots (left), -log10 (*P*-value) of the filtered high-quality SNPs (y-axis) were plotted against their genomic positions (x-axis); SNPs on different chromosomes (chromosomes Z and W were shown as 35 and 36, respectively) were denoted by different colours. The green-red scale at the bottom of Manhattan plot is the marker density. The horizontal black line was present significant genome-wide association threshold. Q–Q plots were displayed as scatter plots of observed and expected log P-values (right) [Colour figure can be viewed at wileyonlinelibrary.com]

weight of the broiler chickens (Sharman et al., 2007). Improving these traits will make chicken well-being better. However, to date, the genetic architectures that underlie the chicken internal organ weights are not completely known. This study preliminarily revealed the genetic architectures of internal organ traits in chickens by performing the estimation of genetic parameters and a GWAS.

4.1 | Estimation of genetic parameters

To figure out the genetic relationships between six internal organ weights in chickens, the estimation of genetic parameters was carried out. As far as the heritabilities of traits were concerned, internal organ weights were moderately to highly heritable. The heritability estimation based on pedigree information for HW, LW and MSW

TABLE 3 Information for significant SNPs and potential candidate genes related to internal organ traits in chickens revealed by GWAS

Trait	N_{snp}	Chr	Position	MAF	Beta	*p*-value	The position of significant SNP in candidate gene
GSW	9	1	157904164	0.47	−0.3799502	9.58E-07	Intergenic region between LOC107051912 (dist = 105.533 kb) and LOC112532749 (dist = 130.85 kb)
		1	160395600	0.368	0.3778163	6.60E-07	Intergenic region between LOC112531551 (dist = 500.809 kb) and PCDH9 (dist = 48.050 kb)
		1	161046470	0.437	−0.3879846	8.95E-07	Intron region of PCDH9
		1	163043553	0.383	0.3763187	6.71E-07	Intergenic region between LOC101747747 (dist = 16.586 kb) and MIR7445−2 (dist = 70.195 kb)
		1	163045840	0.384	0.3738667	7.46E-07	Intergenic region between LOC101747747 (dist = 18.873 kb) and MIR7445−2 (dist = 67.908 kb)
		1	163049234	0.387	0.3709136	6.43E-07	Intergenic region between LOC101747747 (dist = 22.267 kb) and MIR7445-2 (dist = 64.514 kb)
		1	164965900	0.396	0.3743484	5.22E-07	Intergenic region between LOC112531558 (dist = 64.972 kb) and LOC100859897 (dist = 352.491 kb)
		1	170375053	0.417	−0.3974906	3.78E-07	Intron region of FNDC3A
		33	671274	0.061	0.6786905	6.77E-07	Intergenic region between LOC112530878 (dist = 6.58 kb) and LOC112530811 (dist = 7.327 kb)
HW	2	1	170867693	0.071	1.049291	3.26E-07	Intergenic region between LOC101748122 (dist = 40.595 kb) and LOC112531568 (dist = 140.922 kb)
		4	29047258	0.062	1.099683	3.03E-07	Intergenic region between LOC101751121 (dist = 5.18 kb) and NOCT (dist = 146.154 kb)
LW	5	2	11436181	0.317	1.819655	8.06E-07	Intergenic region between LOC112531948 (dist = 424.196 kb) and PFKP (dist = 3.724 kb)
		8	22843864	0.074	2.862713	5.94E-07	Intergenic region between TRABD2B (dist = 23.876 kb) and LOC107054046 (dist = 15.049 kb)
		14	14004856	0.498	−14.27127	7.14E-07	Intron region of ABCA3
		18	8122448	0.069	2.927347	6.46E-07	Intron region of LOC107052241
		19	297731	0.247	1.640454	9.90E-07	Intron region of STX1A
MSW	1	1	179901984	0.344	−1.440623	5.66E-07	Intron region of ZDHHC20
SpW	69	1	170390629	0.236	0.4275662	6.85E-08	Intron region of FNDC3A
		1	170412596	0.237	0.3973435	7.10E-07	Intron region of FNDC3A
		1	170415015	0.237	0.3973435	7.10E-07	Intron region of FNDC3A
		1	170755878	0.464	−0.3316165	7.63E-07	Intron region of LOC107051702
		1	171601904	0.302	0.3649025	3.79E-07	Intron region of TMEM272
		2	31541320	0.255	−0.3666302	4.20E-07	Intergenic region between NPY (dist = 69.272 kb) and MPP6 (dist = 6.599 kb)

(Continues)

TABLE 3 (Continued)

Trait	N_{snp}	Chr	Position	MAF	Beta	*p*-value	The position of significant SNP in candidate gene
		2	45009260	0.134	0.493324	6.76E-07	Intron region of LOC101751579
		2	46475189	0.074	0.6431349	7.05E-07	Intron region of ELMO1
		2	46507938	0.184	0.4143115	9.84E-07	Intron region of ELMO1
		2	75341763	0.053	0.6921145	5.58E-07	Intergenic region between LOC112531784 (dist = 622.805 kb) and BASP1 (dist = 16.492 kb)
		2	75662693	0.081	0.5675726	4.66E-08	Intron region of FAM134B
		3	627	0.089	0.5953208	2.69E-07	Upstream region of RPS27A (dist = 74,194 kb)
		3	35303	0.151	0.3382354	7.15E-07	Upstream region of RPS27A (dist = 39.518 kb)
		3	6382950	0.079	0.6039492	6.54E-07	Intergenic region between OTOR (dist = 715.685 kb) and LOC112532119 (dist = 80.789 kb)
		3	6428933	0.081	0.6352796	2.07E-07	Intergenic region between OTOR (dist = 761.668 kb) and LOC112532119 (dist = 34.806 kb)
		3	6443814	0.083	0.5891414	8.78E-07	Intergenic region between OTOR (dist = 776.549 kb) and LOC112532119 (dist = 19.925 kb)
		3	6451314	0.083	0.5740382	3.43E-07	Intergenic region between OTOR (dist = 784.049 kb) and LOC112532119 (dist = 12.425 kb)
		3	6452251	0.083	0.5681863	5.48E-07	Intergenic region between OTOR (dist = 784.986 kb) and LOC112532119 (dist = 11.488 kb)
		3	6473136	0.082	0.5857608	6.98E-07	Intergenic region between LOC112532119 (dist = 2.979 kb) and LOC112532120 (dist = 26.674 kb)
		3	6485541	0.085	0.6018403	2.97E-07	Intergenic region between LOC112532119 (dist = 15.384 kb) and LOC112532120 (dist = 14.269 kb)
		3	6511094	0.082	0.5904745	9.22E-07	Intron region of LOC112532120
		3	6531497	0.082	0.5634291	9.62E-07	Intergenic region between LOC112532120 (dist = 3.071 kb) and LOC112532121 (dist = 73.255 kb)
		3	6597949	0.08	0.5974599	4.85E-07	Intergenic region between LOC112532120 (dist = 69.523 kb) and LOC112532121 (dist = 6.803 kb)
		3	6599979	0.079	0.6013952	5.22E-07	Intergenic region between LOC112532120 (dist = 71.553 kb) and LOC112532121 (dist = 4.773 kb)
		3	6653200	0.086	0.5894348	3.81E-07	Intergenic region between LOC112532121 (dist = 44.59 kb) and LOC101747264 (dist = 128.916 kb)
		3	6714417	0.086	0.5719207	6.87E-07	Intergenic region between LOC112532121 (dist = 105.807 kb) and LOC101747264 (dist = 67.699 kb)

TABLE 3 (Continued)

Trait	N_{snp}	Chr	Position	MAF	Beta	*p*-value	The position of significant SNP in candidate gene
		3	6718446	0.085	0.6086863	1.58E-07	Intergenic region between LOC112532121 (dist = 109.836 kb) and LOC101747264 (dist = 63.67 kb)
		4	88609435	0.067	0.6081766	9.52E-07	Intron region of PTPRA
		4	89029190	0.056	0.7494076	2.10E-08	Exon region of LOC101750579
		5	10854022	0.052	0.7810328	1.67E-07	Intron region of INSC
		5	11333268	0.053	0.7402796	5.65E-07	Intron region of LOC107053384
		11	20177488	0.361	−0.4559676	2.16E-07	Downstream region of LOC107049584 (dist = 26.911 kb)
		16	2483550	0.394	0.3491633	4.12E-09	Intron region of TRIM7
		16	2500845	0.438	0.3218689	3.79E-08	Exon region of TRIM39.2
		16	2501115	0.406	0.3540305	5.13E-09	Exon region of TRIM27.2
		16	2509404	0.413	0.3419627	1.43E-08	3′UTR region of TRIM27.2
		16	2524251	0.379	0.3757358	1.59E-09	Intron region of TRIM41
		16	2528886	0.342	0.341872	9.57E-09	Intron region of RACK1
		16	2530246	0.406	0.332344	8.90E-09	Intron region of BTN1
		16	2530653	0.427	0.3061707	1.66E-07	Intron region of BTN1
		16	2530981	0.378	0.3385905	1.20E-08	Intron region of BTN1
		16	2531167	0.404	0.3333163	4.10E-09	Intron region of BTN1
		16	2537392	0.408	0.3418201	3.11E-09	Intron region of BTN1
		16	2563450	0.374	0.3369276	3.55E-08	Intron region of BLEC1
		16	2567380	0.365	0.3778442	3.37E-10	Upstream region of BLB1 (dist = 0.419 kb) and Downstream region of TAPBP (dist = 0.448 kb)
		16	2568659	0.403	0.3370953	6.53E-08	Exon region of TAPBP
		16	2576623	0.361	0.3514562	1.05E-08	Intron region of BRD2
		16	2577923	0.361	0.3482862	1.36E-08	Intron region of BRD2
		16	2578661	0.384	0.3480763	8.53E-09	Exon region of BRD2
		16	2578982	0.361	0.3524173	8.69E-09	Intron region of BRD2
		16	2579544	0.407	0.3432755	2.53E-09	Intron region of BRD2
		16	2587381	0.373	0.3277378	7.82E-08	Exon region of DMB1
		16	2588182	0.454	0.3330131	8.91E-09	Exon region of DMB1
		16	2598840	0.418	0.3111921	5.34E-08	Intron region of TAP1
		16	2599123	0.39	0.3101257	1.88E-07	Exon region of TAP1
		16	2599422	0.444	0.327946	2.35E-08	Intron region of TAP1
		16	2600046	0.362	0.3480421	1.36E-08	Exon region of TAP1
		16	2610458	0.453	0.3328535	1.18E-08	Exon region of C4
		16	2612708	0.39	0.3405395	1.09E-08	Intron region of C4
		16	2613998	0.387	0.3501272	3.58E-09	Intron region of C4
		16	2616717	0.396	0.3323223	3.77E-08	Exon region of C4
		16	2620131	0.421	0.3230829	1.80E-08	Intron region of C4
		16	2630673	0.385	0.3327989	8.10E-09	Exon region of CYP21A1
		16	2634329	0.37	0.329086	1.71E-08	Intron region of TNX
		16	2634660	0.378	0.3407062	4.36E-09	Intron region of TNX

(Continues)

TABLE 3 (Continued)

Trait	N_{snp}	Chr	Position	MAF	Beta	*p*-value	The position of significant SNP in candidate gene
		16	2649332	0.421	0.332905	1.45E-08	Splicing site of TNX
		16	2651138	0.396	0.336273	5.02E-09	Exon region of TNX
		16	2657664	0.401	0.3507334	5.35E-10	Upstream region of LOC101751902 (dist = 1.248 kb)
		27	5335143	0.063	0.6802002	8.12E-08	Intron region of KANSL1
TW	17	1	96567610	0.221	3.816551	5.67E-07	Intron region of GBE1
		1	96603222	0.283	3.736372	6.38E-07	Intron region of GBE1
		1	97110674	0.234	3.872294	4.10E-07	Intergenic region between LOC101748186 (dist = 390.435 kb) and LOC101748284 (dist = 99.737 kb)
		1	97346214	0.254	3.738403	9.51E-07	Intron region of ROBO1
		1	97517953	0.248	3.807213	7.34E-07	Intron region of ROBO1
		1	97769053	0.254	3.798404	6.18E-07	Intron region of ROBO1
		1	97857465	0.281	3.6835	9.73E-07	Intron region of ROBO1
		1	97872500	0.252	3.745499	7.59E-07	Intron region of ROBO1
		7	20471018	0.426	3.476731	9.23E-07	Intergenic region between LOC101749123 (dist = 227.814 kb) and LOC107053823 (dist = 16.943 kb)
		7	20472562	0.426	3.476731	9.23E-07	Intergenic region between LOC101749123 (dist = 229.358kb) and LOC107053823 (dist = 15.399 kb)
		7	21768144	0.461	3.367013	6.85E-07	Downstream region of RBMS1 (dist = 1.554 kb)
		7	22695888	0.351	3.612059	2.19E-07	Intergenic region between LOC112532799 (dist = 16.868 kb) and LOC101749755 (dist = 68.1 kb)
		7	23458496	0.283	3.82037	9.65E-07	Exon region of LOC107053887
		7	23460051	0.283	3.82037	9.65E-07	Exon region of LOC107053887
		7	23463551	0.283	3.82037	9.65E-07	Intron region of LOC107053887
		7	23464459	0.283	3.82037	9.65E-07	Intron region of LOC107053887
		7	23464738	0.283	3.82037	9.65E-07	Intron region of LOC107053887

Abbreviations: Beta, the estimate coefficient; Chr, chromosome; GSW, glandular stomach weight; HW, heart length; LW, liver weight; MAF, minor allele frequency; MSW, muscular stomach weight; Nsnp, number of significant; SNP, retaining the most significant one if the distance between multiple SNPs of the same trait is less than 0.25 Mb; SpW, spleen weight; TW, testis weight.

were 0.38, 0.25 and 0.39 in one study (Gaya et al., 2006), as well as 0.27, 0.33 and 0.44 in another study (Venturini et al., 2014). The heritability estimation for HW (0.283), LW (0.355), GSW (0.408) and MSW (0.640) were reported based on REML (Dou et al., 2019). In general, heritabilities of these internal organ traits were reported to be moderate to high, which was in accordance with our results. Investigating genetic and phenotypic correlations is an effective way to mirror the relationships between the traits of interest. Our data revealed that there were significantly positive genetic and phenotypic correlations between most of them. The study by Venturini et al. (2014) revealed that the genetic correlation coefficients between MSW, HW and LW, were positive, ranging from 0.38 to 0.56. In the study by Dou et al. (2019) the genetic correlation coefficients between internal organ weights (MSW, GSW, HW and LW) were positive, varying from 0.503 to 0.711. These results are similar to our data. However, the study by Gaya et al. (2006) showed that there were negative genetic correlations between MSW and HW (−0.02), and between MSW and LW (−0.11). The differences in genetic parameters are mainly attributed to different estimation methods, sample sizes and the genetic background of populations in these studies.

TABLE 4 Overlaps between the detected genes herein, and published QTL for internal organ traits in chickens based on GG6.0 reference genome

Traits	Genes	Chr	Gene position (bp)	Known QTL[a]
GSW, SpW	fibronectin type III domain containing 3A (*FNDC3A*)	1	170,318,524–170,431,952	QTL# 170493 QTL # 170497
HW	*LOC101748122*	1	170,806,199–170,827,098	QTL # 170491 QTL # 170495
SpW	membrane palmitoylated protein 6 (*MPP6*)	2	31,547,919–31,603,914	QTL # 1886; QTL #12571
SpW	*LOC107049584*	11	20,149,824–20,150,577	QTL # 2287
SpW	KAT8 regulatory NSL complex subunit 1 (*KANSL1*)	27	5,290,867–5,358,306	QTL # 21756

Abbreviations: GSW, glandular stomach weight; HW, heart weight; SpW, spleen weight.

[a] Chicken QTL db ID numbers database–release 45.

4.2 | GWAS

The purpose of F_2 design is to generate larger genetic variation and trait segregation through the DNA recombination, which is beneficial to QTL mapping (Yuan et al., 2015). In this study, the F_2 population was constructed by crossing broiler cocks derived from Arbor Acres with high abdominal fat content and Baier layer dams (a Chinese native breed). Descriptive statistics displayed that the coefficient of variation (from 18.81% to 69.44%) and standard deviation (from 1.03 to 7.54) of internal organ weights was large, indicating that there was good segregation of traits and a large diversity of phenotypic traits within the population, which will benefit QTL mapping for internal organ weights.

Most significant SNPs responsible for internal organ traits were newly identified and were scattered on different chromosomes of the chicken. A few genomic regions comprised several QTLs that were reported to associate with GSW, SpW and HW based on Chicken QTL database–release 45 (Table 4) (Hu et al., 2019). Besides, we found that *FNDC3A* was associated with GSW and SpW (Table 4), known as pleiotropy, which is a pervasive phenomenon in the genetic architecture of domestication in chickens (Wright et al., 2010). This could also be confirmed by our data that there are highly positive genetic correlations amongst most of these internal organ traits in this study.

4.3 | Candidate gene function

We examined the functional annotation and physical position of these genes to identify potential candidate genes that may impact the weights of internal organs. Of all the PCGs we revealed, major histocompatibility complex, class II, DM beta 1 (*DMB1*) and major histocompatibility complex class II beta chain BLB1 (*BLB1*) had been previously reported as candidate genes for regulation of internal organ weights in chickens in two recent studies. *DMB1* and *BLB1* genes were only expressed at the highest levels in the spleen and intestine by differential tissue-specific expression study (Parker & Kaufman, 2017). Guo, Su, et al. (2020) and Guo, Jiang, et al. (2020) speculated that *DMB1* might play an important role in the occurrence of stress-induced immunosuppression because it was strongly activated in the Dex-induced spleen of chicks by transcriptomic analysis of the spleen. *BLB1* play important roles in response to Salmonella enterica serovar Enteritidis (SE) inoculation (Wu et al., 2015). Two underlying PCGs phosphofructokinase, platelet (*PFKP*) and roundabout guidance receptor 1 (*ROBO1*) were functionally confirmed to be associated with internal organ weights in humans and mice respectively. High PFKP mRNA expression was associated with the worst stages of liver cancer in humans (Zhang et al., 2020). *ROBO1* play an important role in the regulation of testis function in mice and an increase in intra-testicular testosterone concentrations was found in Robo1-null mice (Martinot & Boerboom, 2021).

These pieces of evidence showed that these genes were plausible candidates responsible for the growth and development of internal organs in chickens. In most cases, chromosome 1 was found to be strongly associated with internal organs at different ages in various breeds of chickens. The identified genes associated with internal organ traits in this study were not entirely consistent with previous GWAS results (Dou et al., 2019; Moreira et al., 2019), which are probably due to many factors such as breeds, ages, sample size and so on (Dou et al., 2019; Visscher et al., 2017). It is necessary to experimentally verify our findings by investigating the effects of these identified candidate genes on internal organs in the future. In summary, our results provide a useful reference for fine mapping causative genes and SNPs responsible for chicken internal organ weights.

5 | CONCLUSION

We performed genetic parameter estimation and a GWAS in an F_2 chicken population to dissect genetic architectures underlying internal organ weights. The weights of six tested internal organs showed moderate to high heritability, and there were positive genetic and phenotypic correlations between most of them. The GWAS results exhibited that an array of genes and many novel QTL regions associated with internal organ weight traits in this study.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

DATA AVAILABILITY STATEMENT

None of the data were deposited in an official repository. Data may be available upon request by contacting the corresponding author.

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