Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2024, Volume 4, Issue 1, Page No: 49-60

Available online at: www.esvpub.com/

Genome-Wide CNV Mapping and GWAS Identify Candidate Genes for Body Weight and Egg Quality in Wenshui Green Shell-Laying Chickens

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ABSTRACT

Copy number variation (CNV) represents a key form of genetic diversity that can influence gene regulation, phenotypic variability, disease predisposition, and evolutionary processes in animals. To gain deeper insights into the weight and egg quality characteristics of chickens, this study aimed to detect CNVs in Wenshui green-shelled laying chickens and perform a genome-wide association study (GWAS) based on copy number variation regions (CNVRs). The goal was to identify genetic variants and candidate genes correlated with weight and egg quality traits to aid breeding improvements. In total, 11,035 CNVRs were identified in Wenshui green-shelled laying chickens, covering 13.1 Mb, approximately 1.4% of the autosomal genome. Among these CNVRs, 10,446 were of the loss type, 491 were gains, and 98 were mixed types. Notably, two CNVRs were significantly linked to egg quality, while four showed associations with body weight, all located on chromosome 4. Candidate genes potentially related to these traits included FAM184B, MED28, LAP3, ATOH8, ST3GAL5, LDB2, and SORCS2. This study constructed the first CNV map of the Wenshui green-shelled chicken genome using population genotyping, suggesting that CNVRs can serve as molecular markers to enhance weight and egg quality traits in chicken breeding.

Keywords: Wenshui green-shelled chicken, Weight and egg quality, Copy number variation, Genome-wide association study

Received: 19 October 2023
Revised: 17 January 2024
Accepted: 21 January 2024

How to Cite This Article: Xiu J, Jian F, Xin W. Genome-Wide CNV Mapping and GWAS Identify Candidate Genes for Body Weight and Egg Quality in Wenshui Green Shell-Laying Chickens. Int J Vet Res Allied Sci. 2024;4(1):49-60.
https://doi.org/10.51847/6ZQUxn08QL

Introduction

Copy number variation (CNV) is a common structural genomic alteration, ranging in size from 50 bp to several Mb, and includes deletions, insertions, recombinations, and complex multi-site mutations [1]. CNV plays a crucial role in the evolution of phenotypic diversity and population adaptability [2], accounting for a large proportion of total genetic variability within species [3]. It introduces gene structural changes mainly through dosage and positional effects [4, 5], influencing organismal flexibility and disease progression [6]. CNVs are abundant across human and animal genomes, spanning more nucleotides than single-nucleotide polymorphisms (SNPs) and substantially expanding genetic diversity [7].

In poultry breeding, genetic variation has become a major focus as one of the main determinants of production traits. Multiple studies have reported CNVs related to broiler body weight [8], abdominal fat [9], and skin pigmentation [10], as well as breed-specific CNVs identified in populations [11]. Thus, studying chicken traits at the genomic level provides valuable insight into the improvement of economic performance.

A variety of techniques are available to study CNV. Apart from conventional cytogenetic methods, approaches such as Array Comparative Genomic Hybridization (aCGH) [12], SNP chip analysis [13], and next-generation

sequencing (NGS) [14] have been applied. Compared with aCGH and SNP arrays, NGS offers higher resolution, broader detection capacity, and the ability to assess genetic diversity [15]. CNV detection from whole-genome sequencing (WGS) data can be conducted using various algorithms, which are categorized as Read-pair (RP), Split-read (SR), Read-depth (RD), Assembly (AS), or Combined Approach (CA) tools [16, 17].

With the expansion of CNV research in animals, CNV-based GWAS has been increasingly applied [2, 8, 18–21]. Since the concept of GWAS was introduced by Risch *et al*. (1996) [22], it has been widely utilized to identify genes associated with human genetic disorders [23]. As sequencing technologies evolved, the focus of CNV-GWAS gradually extended from human diseases to economically relevant traits in livestock [18, 19, 24–26]. This suggests that CNVs could play an essential role in shaping critical economic characteristics in farm animals [27]. The objective of this study was to detect CNVs in Wenshui green-shelled laying chickens and to perform CNVR-based GWAS to investigate their association with weight and egg quality traits. The results are expected to provide genetic insights and identify candidate genes contributing to these traits, laying a foundation for applying molecular breeding methods such as marker-assisted selection and genomic selection to improve poultry performance.

Materials and Methods

Population description

The chicken population used in this study was obtained from Jinqiu Agricultural and Animal Husbandry Technology Co., Ltd. (Tai'an, China). A total of 834 Wenshui green-shelled laying chickens from the same generation were selected. Egg production data were collected for three months after the onset of laying, while egg quality measurements were taken at 30 and 40 weeks of age. The experimental population represents an improved breed developed by crossing Wenshang reed-feather chickens with Xinyang green-shelled chickens. Through selective breeding combined with molecular detection techniques, this line was developed to integrate favorable traits such as reed feather pattern, green eggshells, high egg yield, and superior egg quality.

Phenotyping

At 30 weeks of age, the egg weight (EW) and egg shape index (ESI) of Wenshui green-shelled laying chickens were recorded. At 40 weeks, additional egg quality parameters were measured, including yolk color (YC), egg white height (EWH), shell thickness (SH), shell strength (SS), yolk weight (YW), shell weight (SW), egg white weight (EWW), yolk ratio (YR), shell ratio (SR), egg white ratio (EWR), concentrated egg white long diameter (EWL), concentrated egg white short diameter (EWS), and the Haugh unit (HU). The body weights of the chickens were collected at birth (BW) and at 4, 8, 13, 15, and 38 weeks of age. All phenotypic data underwent quality control testing to ensure reliability.

Blood samples were drawn from the test group at approximately 50 weeks of age through the subwing vein. Before sampling, insulation was arranged to prevent sample degradation caused by high temperatures, and the samples were stored at −20 °C. Genomic DNA was extracted using TLANGEN's Genomic DNA Extraction Kit, following the phenol–chloroform method. DNA integrity was confirmed through 1% agarose gel electrophoresis [28]. DNA concentration and purity were determined using the OD260/OD280 ratio, which ranged between 1.7 and 1.9. High-quality DNA samples were then sent to Beijing Youji Technology Co., Ltd. (Beijing, China) for whole-genome sequencing (paired-end sequencing, 150 bp read length) to produce the raw genomic dataset.

Sequence alignment to reference genome

Quality filtering of raw reads was conducted with Trimmomatic v0.38 [29], removing adapter sequences. After filtering, each sample contained an average of 44,931,409 reads, with a mean sequencing depth of 11.74×. The processed reads were aligned to the Wenshui green-shelled chicken reference genome using bwa v0.7.17 [30]. The mean mapping rate reached 99.76%, while the average genome coverage was 97.55%. Repetitive elements were subsequently marked using GATK v4.2.6.1 [31].

CNV detection

The DELLY v1.1.6 program was applied using the Combined Approach (CA) to detect CNVs within the population genome data. This tool integrates paired-end and split-read mapping to identify structural variations—such as deletions, duplications, inversions, and translocations—with high precision and sensitivity [32]. Since

DELLY is optimized for population-based CNV detection, overlapping CNVs across individuals were merged into copy number variation regions (CNVRs), defined as large genomic segments where adjacent CNVs overlap [33]. CNVRs were classified as gain, loss, or mixed types (if both occurred in the same region).
Filtering retained only entries labeled "PASS" in the FILTER column of the VCF file. CNVRs between 50 bp and 5 Mb in size were kept for further analysis. Subsequently, BEDTools v2.26.0 was used to merge overlapping CNVs across samples, ensuring accurate CNVR construction [34].

CNV-based GWAS
Only CNVRs with a population frequency above 0.5% were used to enhance the accuracy of GWAS outcomes [18]. The processed VCF files were converted into .bim, .fam, and .bed formats using PLINK v2.0 [35]. In these datasets, genotypes were encoded as follows: gain = 1, loss = −1, and normal (2n) = 0 [8, 19]. A mixed linear model (MLM) implemented in GMAT software was used to perform single-trait GWAS [36]. All birds were from the same hatch period and reared under identical conditions in tiered cages. The statistical model used was:

$$y = \mu + Wg + Zu + e \quad (1)$$

where y represents the phenotypic observation vector; μ is the overall mean; g is the vector of CNV effects, with W as its design matrix; u represents polygenic effects, and Z is its design matrix; e denotes random residuals.
Random effects were assumed to follow a normal distribution:

$$u \sim N\left(0, G\sigma\frac{2}{a}\right), e \sim N\left(0, I\sigma\frac{2}{e}\right) \quad (2)$$

where G is the genomic relationship matrix derived from CNV data, I is the identity matrix, σ^2_a is the additive genetic variance, and σ^2_e is the residual variance. The genome-wide significance threshold was determined as (0.05/N), where N is the total number of CNVRs analyzed [18].

Gene annotation
Custom scripts were used to identify genes located within a 100 kb window (±50 kb upstream and downstream) surrounding the significant CNVRs, based on the GFF file of the Wenshui green-shelled chicken reference genome.

Results and Discussion

Quantity and chromosomal distribution of CNVRs
Copy number variation analysis was conducted on the same group of 834 Wenshui green-shelled laying hens using DELLY software version 1.1.6. After merging overlapping CNVRs among all samples, a total of 11,035 CNVRs were identified, covering 13.1 Mb, which corresponds to approximately 1.4% of the chicken genome. Among these, 10,446 were classified as loss variants, 491 as gain variants, and 98 exhibited both loss and gain events in the same genomic region. The CNVRs ranged in size from 51 bp to 642.6 kb, with a mean length of 1.2 kb.
Within the autosomes, chromosome 1 contained the greatest number of CNVRs (2761), while chromosome 35 showed the fewest (2). No CNVRs were found on chromosomes 29, 32, or 37, which may be attributed to differences in chromosome length. The overall CNVR distribution among autosomes is illustrated in **Table 1**, **Figures 1 and 2**.

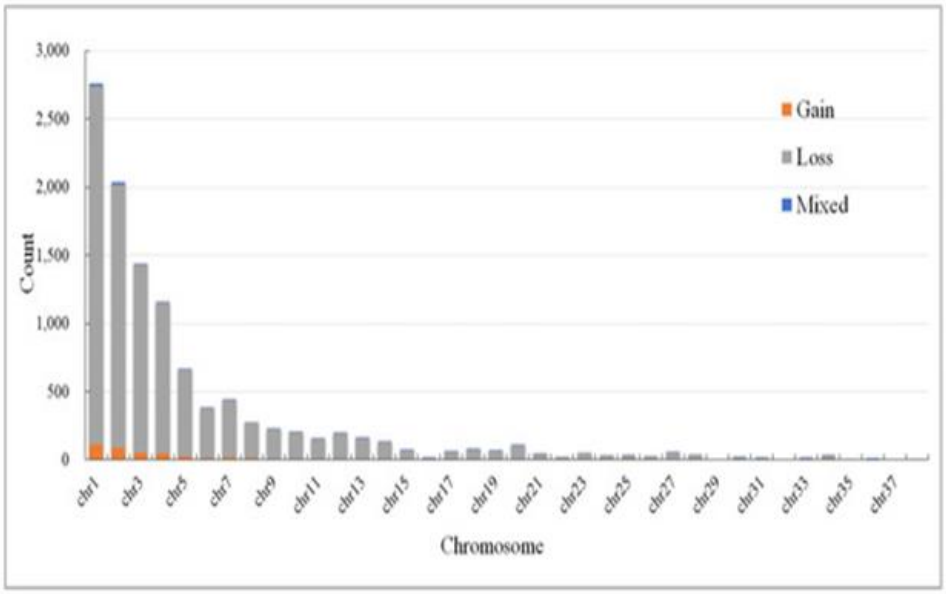


Figure 1. Spatial distribution of CNVR categories in Wenshui green-shelled laying chickens.

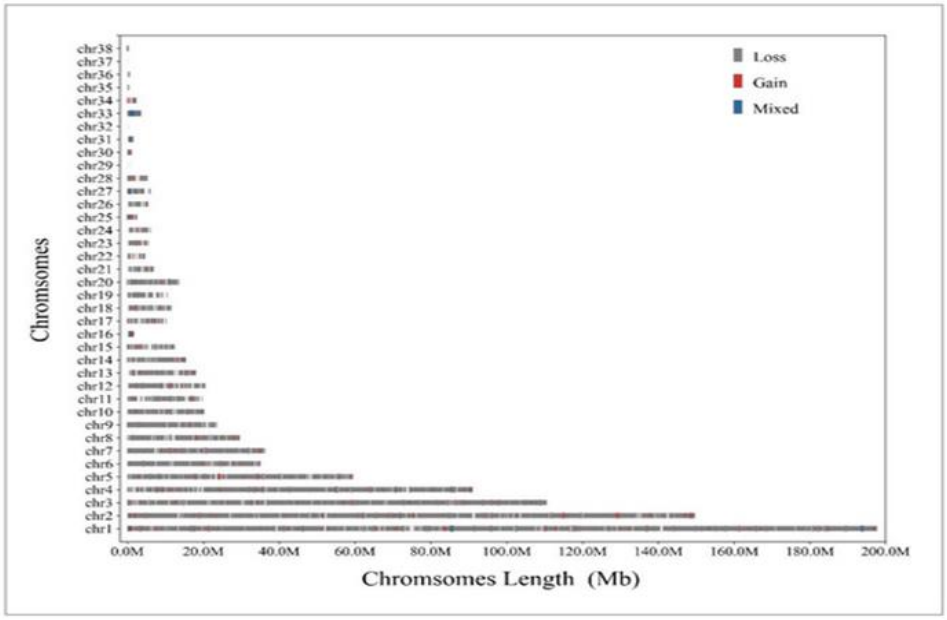


Figure 2. Genome-wide CNVR maps across 38 autosomes, showing three variation types: loss (grey), gain (red), and mixed (blue). The Y-axis indicates autosomes, and the X-axis represents chromosomal positions in Mb.

Table 1. Distribution of CNVRs among autosomal chromosomes of the Wenshui green-shelled chicken genome.

Chr	Chr Length (bp)	CNVR Count	CNVR Total Length (bp)	Coverage (%)	Max CNVR Size (bp)	Average CNVR Size (bp)	Min CNVR Size (bp)
1	197,778,178	2761	4,102,658	2.1	642,753	1485.9	51
2	149,541,958	2038	1,795,942	1.2	76,049	881.2	51
3	110,815,227	1435	1,011,600	0.9	37,549	704.9	51
4	91,021,375	1162	894,849	1.0	104,295	770.1	51
5	59,471,259	664	581,860	1.0	38,456	876.3	51
6	35,339,061	382	241,170	0.7	21,044	631.3	51

7	36,318,844	437	353,483	1.0	36,899	808.9	51
8	29,613,760	276	143,465	0.5	20,456	519.8	52
9	23,556,363	224	177,489	0.8	15,124	792.4	51
10	20,214,400	204	122,553	0.6	12,825	600.8	51
11	19,755,808	157	81,894	0.4	16,494	521.6	51
12	20,438,972	194	124,811	0.6	19,162	643.4	51
13	18,437,548	161	285,249	1.5	50,758	1771.7	51
14	15,523,295	135	89,883	0.6	18,875	665.8	51
15	12,662,000	71	80,617	0.6	14,389	1135.5	51
16	1,595,800	11	615,070	38.5	542,075	55,915.5	63
17	10,229,956	57	22,414	0.2	4896	393.2	51
18	11,472,971	78	101,581	0.9	35,771	1302.3	51
19	10,411,340	66	65,454	0.6	12,765	991.7	51
20	14,040,156	110	78,193	0.6	16,021	710.8	51
21	6,776,000	42	36,126	0.5	27,274	860.1	52
22	4,690,381	23	15,414	0.3	4663	670.2	53
23	5,830,993	53	17,315	0.3	6364	326.7	52
24	6,352,200	37	16,508	0.3	6088	446.2	53
25	2,575,857	34	44,705	1.7	10,788	1314.9	52
26	5,288,600	30	23,279	0.4	13,044	776.0	51
27	5,930,361	56	425,849	7.2	311,709	7604.4	53
28	5,407,282	42	29,718	0.5	12,414	707.6	52
29	1,064,585	0	0	0	0	0	0
30	979,082	21	8930	0.9	1139	425.2	54
31	2,139,823	13	308,985	14.4	193,165	23,768.1	58
32	454,000	0	0	0	0	0	0
33	3,524,363	19	1,142,884	32.4	504,390	60,151.8	52
34	2,223,258	30	28,054	1.3	16,716	935.1	73
35	327,777	2	17,147	5.2	15,351	8573.5	1796
36	493,600	7	7529	1.5	3094	1075.6	226
37	316,000	0	0	0	0	0	0
38	298,400	3	1258	0.4	477	419.3	365
Overall	**6,247,088**	**11,035**	**13,093,936**	**1.4**	**642,753**	**1186.6**	**51**

Out of the total 11,035 CNVRs, 9645 (87.4%) were within the 0.05–5 kb range, followed by 1060 (9.6%) within 1–5 kb, 146 (1.3%) within 5–10 kb, 160 (1.5%) within 10–50 kb, and 24 (0.2%) larger than 50 kb **(Figure 3)**.

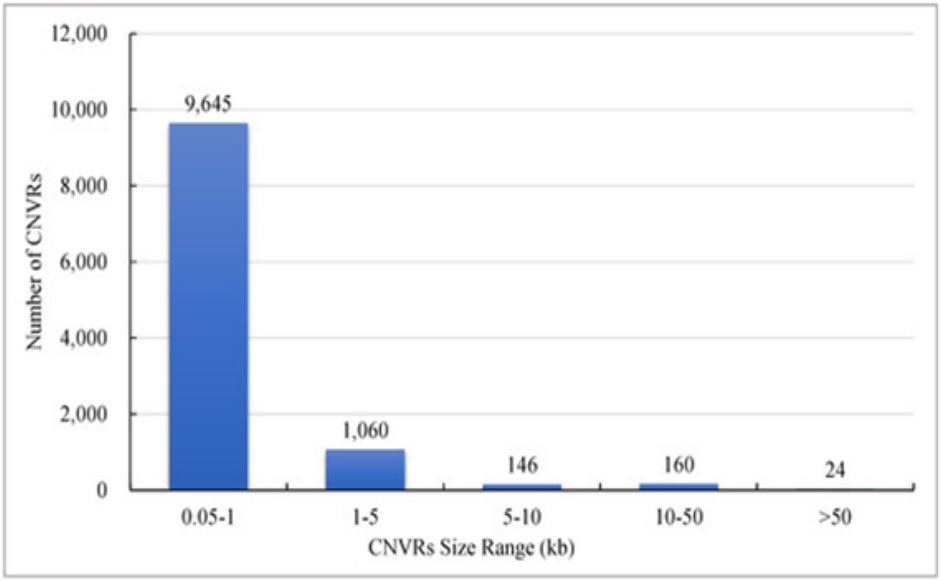
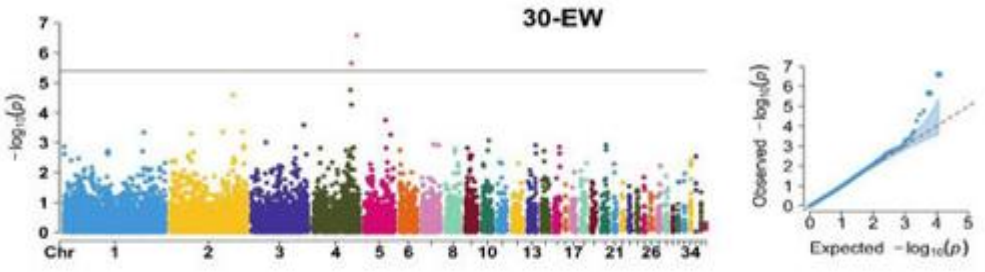


Figure 3. Distribution of CNVR lengths in Wenshui green-shelled laying chickens.

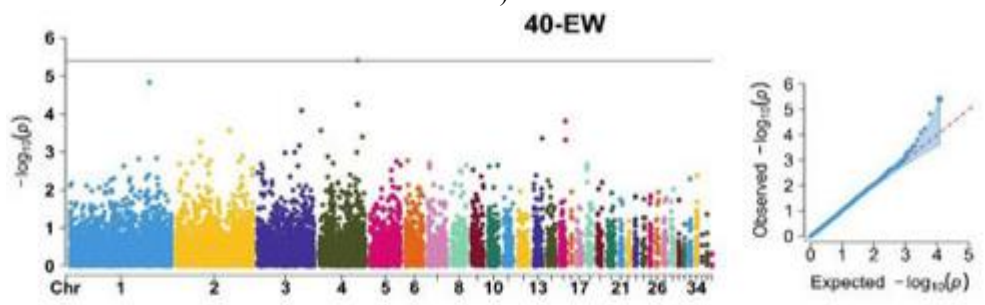
Genome-wide association analysis based on CNVRs

A genome-wide association study (GWAS) was conducted to detect links between CNVRs and 23 traits related to body weight and egg quality in the Wenshui green-shelled chicken population. Five traits demonstrated statistically significant associations with CNVRs. A Manhattan plot displaying these associations across the 38 autosomes is shown in **Figure 4**.

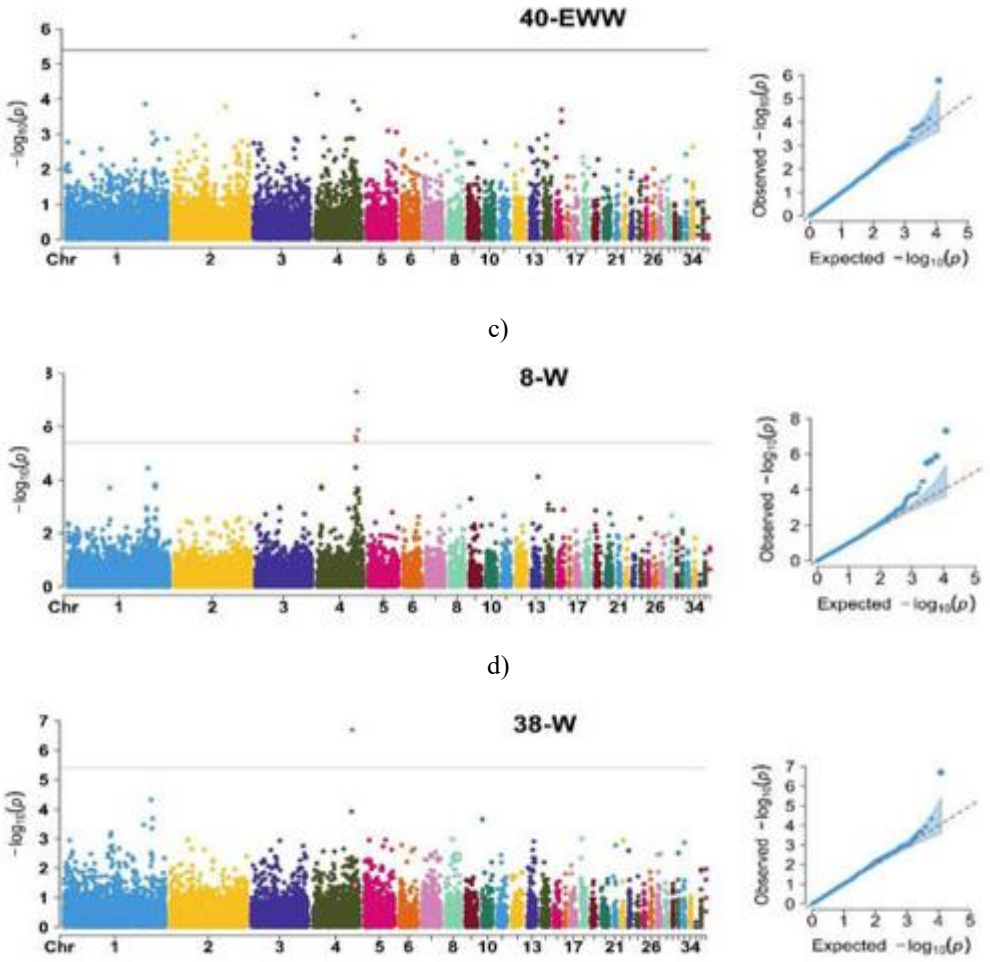
Table 2 summarizes the CNVRs significantly associated with weight and egg characteristics. Among them, two CNVRs were linked to 30-EW, one CNVR to 40-EW and 40-EWW, four CNVRs to 8-W, and one CNVR to 38-W. Within the 100-kb flanking regions of these significant CNVRs, eleven genes were identified that may be functionally related to 30-EW, 40-EW, 40-EWW, 8-W, and 38-W.



a)



b)



c)

d)

e)

Figure 4. Manhattan and QQ plots showing CNVR segments on 38 autosomes associated with 30-EW, 40-EW, 40-EWW, 8-W, and 38-W. The X-axis represents chromosome number, while the Y-axis shows −log10(p-value). The plotted horizontal lines mark the FDR-adjusted significance level (0.05). The Manhattan plot highlights significant CNVR–trait associations, and the QQ plot displays their statistical distribution.

Table 2. CNVRs are significantly associated with body weight and egg quality traits in Wenshui green-shelled chickens.

Trait[1]	CNVR ID	Type[2]	Chromosome	CNVR Position (bp)[3]	p-Value[4]	Proximal Gene(s)[5]
30-EW	DUP00035918	Gain	4	85,203,669–85,205,350	2.52×10^{-7}	*ATOH8 ST3GAL5*
30-EW 40-EW 40-EWW	DEL00035336	Loss	4	75,882,077–75,882,619	2.22×10^{-6} 3.93×10^{-6} 0.65×10^{-6}	*FAM184B MED28 LAP3*
8-W	DEL00035339	Loss	4	76,019,681–76,020,779	2.35×10^{-6}	*LOC112532307 LDB2*
8-W 38-W	DEL00035404	Loss	4	77,124,281–77,124,469	5.01×10^{-8} 2.02×10^{-7}	*LOC107053295*
8-W	DEL00035425	Loss	4	77,492,948–77,493,042	3.16×10^{-6}	*LOC121110716*
8-W	DEL00035578	Loss	4	80,144,379–80,145,908	1.35×10^{-6}	*SORCS2 LOC121110591*

1. 30-EW: egg weight at 30 weeks; 40-EW: egg weight at 40 weeks; 40-EWW: egg white weight at 40 weeks; 8-W: body weight at 8 weeks; 38-W: body weight at 38 weeks.

2. Gain: duplication events; Loss: deletion events.
3. CNVR Position: genomic coordinates according to the Wenshui green-shelled chicken reference genome.
4. p-value: genome-wide significance threshold.
5. Proximal Gene: gene ID according to the NCBI database.

In the present study, 11,035 CNVRs were identified in Wenshui green shell-laying chickens, spanning a combined length of 13.1 Mb, which accounts for roughly 1.4% of their autosomal genome. These included 10,446 deletions, 491 duplications, and 98 mixed variations, with CNVR lengths ranging between 51 bp and 642.6 kb.

When compared with earlier research, for instance, Rao *et al*. reported 357 CNVRs detected through PennCNV in F_2 populations of White Recessive Rock and Xinhua chickens, consisting of 213 deletions, 112 duplications, and 32 mixed types [37]. Chen *et al*. integrated several algorithms—mrFAST, CNVnator, BreakDancer, and Pindel—to analyze CNVs among one original breed (Red Jungle fowl), two commercial breeds (Recessive White Rock and White Leghorn), and several Chinese local breeds (Xinghua, Luxi Game fowl, and Beijing-You). They identified 11,123 CNVRs, including 8834 loss types, 1911 gain types, and 378 mixed types [38]. Likewise, Seol *et al*., using CNVnator v0.4, detected 3079 CNVRs in four breeds (Cornish, White Leghorn, Rhode Island Red, and Red Jungle fowl), of which 2443 were losses and 636 were gains [11].

In another work, Zhang *et al*. applied PennCNV to broiler lines of the 475th generation from Northeast Agricultural University, detecting 460 CNVRs—320 losses, 93 gains, and 47 mixed forms [9]. Han *et al*. employed aCGH microarray analysis in five chicken breeds (Xichuan black-bone, Silkie, Lushi, Gushi, and Houdan) and found 281 CNVRs, composed of 181 losses, 91 gains, and 9 mixed types [10].

Across studies, CNVR overlap rates were relatively low, likely influenced by multiple variables such as differences in technological platforms (aCGH, SNP array, or NGS), species diversity, software algorithms, and data filtering criteria. In this work, loss-type CNVs were notably more abundant than gain or mixed types, aligning with trends reported in previous investigations. While no definitive cause has been established, these differences may stem from genetic structural variation among breeds and distinct selective pressures during breeding.

Additionally, the absence of CNVs on certain chromosomes may result from several technical or biological factors, including genome assembly quality, methodological limitations, and chromosomal architecture. Given that genomic quality control was thoroughly conducted and the detection method applied here is broadly validated, it is plausible that intrinsic chromosomal structure explains the observed gaps.

Following a CNVR-based GWAS for body weight and egg quality parameters, six CNVRs located on chromosome 4 exhibited significant associations with these traits. Among them, two CNVRs correlated with egg quality and involved the genes FAM184B, MED28, LAP3, ATOH8, and ST3GAL5, while four CNVRs associated with body weight traits contained LOC112532307, LDB2, LOC107053295, LOC121110716, SORCS2, and LOC121110591.

The FAM184B gene, a protein-coding locus expressed in multiple tissues including skin and brain, was previously linked by Zhang *et al*. to initial laying weight in Jinghai Yellow chickens [39], and by Jin *et al*. to body weight in Yancheng chickens [40]. In the current analysis, 30-EW and 40-EW were associated with FAM184B. Since hen body weight at sexual maturity strongly influences egg weight at onset and peak laying [41–43], it is inferred that FAM184B indirectly modulates egg mass by affecting growth traits in Wenshui green shell-laying chickens.

MED28, another protein-coding gene, is implicated in cell cycle control and proliferation. Previous studies revealed associations of MED28 with body mass and intramuscular fat in cattle [44–46], as well as prenatal and postnatal weight in sheep [47, 48], and muscle development in pigs [49]. No prior data have reported its function in chickens. In this research, correlations between 30-EW and 40-EW with MED28 were found, suggesting it may affect egg weight indirectly through modulation of body weight.

The LAP3 gene encodes an aminopeptidase involved in protein degradation and maturation [50]. Liu *et al*. demonstrated its relationship with carcass and visceral weight in Beijing-You chickens [51]. Here, 30-EW and 40-EW were also correlated with LAP3, supporting its indirect influence on egg weight through effects on body growth in Wenshui chickens.

ATOH8, a bHLH transcription factor, contributes to the development of nervous tissue, pancreas, muscle, retina, and kidney [52]. Its expression has been observed during chicken skeletal myogenesis [53, 54], and in mice, ATOH8 regulates myoblast proliferation by influencing myopeptide signaling [53]. Given that muscle metabolic status plays a major role in determining egg quality [55], the identified link between 30-EW and ATOH8 implies that this gene may influence egg weight indirectly through skeletal muscle growth in Wenshui green shell-laying chickens.

The ST3GAL5 gene encodes a protein involved in glycosylation pathways, contributing to immune regulation and nervous system formation [56, 57]. Studies on chickens demonstrated that ST3GAL5 acts on lactosylceramide and shows relatively strong expression in the small intestine, large intestine, and spleen [58]. Both intestinal regions are essential for maintaining digestive, metabolic, endocrine, and immune activities in poultry and livestock [59]. In the present research, a connection between egg weight at 30 weeks (30-EW) and ST3GAL5 was observed, implying that this gene might modulate egg weight in Wenshui green shell-laying chickens through its influence on the digestive system.

The LDB2 gene, known to interact with several transcription factors, plays a pivotal role in neural development and angiogenesis [60, 61]. Gu *et al*. identified a link between LDB2 on chromosome 4 and body mass during weeks 7–12 in an F_2 population derived from Silky fowl and White Plymouth Rock [62]. Similarly, Zhang *et al*. and Wang *et al*. reported associations of LDB2 on the same chromosome with body weight in Gushi-Anka F_2 and Jinghai Yellow hens [63, 64]. Liu *et al*. also found a relationship between LDB2 and carcass and visceral weight in Beijing-You chickens [51], while Dou *et al*. regarded it as a candidate gene responsible for rapid growth in broilers [65]. In this study, LDB2 was linked with 8-week body weight (8-W), indicating its impact on growth performance in Wenshui green shell-laying chickens.

The SORCS2 gene belongs to the Vps10p-domain receptor family, which is connected to neurological conditions in mammals. In chickens, Li *et al*. explored the genetic control of aggressive traits and showed that SORCS2 on chromosome 4 in Chinese native dwarf yellow chickens may regulate dopaminergic pathways and neurotrophic mechanisms tied to aggressive behavior [66]. Chen *et al*. further supported SORCS2 as a candidate gene for aggressiveness in Luxi Game fowl [38]. In this work, a relationship between 8-W and SORCS2 was identified, implying that SORCS2 may influence growth-related traits in Wenshui green shell-laying chickens.

This study represents the first CNV characterization in Wenshui green shell-laying chickens. CNVs were detected and merged into CNVRs, focusing only on autosomal chromosomes. A GWAS was then applied to uncover relationships between CNVRs and weight and egg quality traits. Interpreting genes neighboring significant CNVRs provides insight into trait development in this breed. Nevertheless, it is important to note that multiple genetic factors—including SNPs, SVs, CNVs, and DNA methylation—influence such phenotypes. Since this study solely addressed CNV variation, further investigation encompassing other genomic features would enhance the overall genetic understanding of the Wenshui green shell-laying chicken.

Conclusion

For the first time, CNVs in Wenshui green shell-laying chickens were detected and grouped into CNVRs. A GWAS based on these CNVRs examined their links to weight and egg characteristics. Altogether, 11,035 CNVRs were found, covering around 1.4% of the autosomal genome. The genes FAM184B, MED28, LAP3, ATOH8, and ST3GAL5 emerged as candidates influencing 30-EW and 40-EW, whereas LDB2 and SORCS2 were linked to 8-W. These findings underscore the potential role of CNVs in shaping weight and egg quality traits, offering new genomic perspectives for future genetic improvement studies in this breed.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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