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Authors' contributions Please specify the authors' role using this form.	Conceptualization: Kim J, Choo H, Lee JH. Data curation: Kim J, Choo H. Formal analysis: Kim J. Methodology: Kim J, Cho E, Kim M Software: Kim J, Cho E. Validation: Kim J, Kim M, Kim J. Investigation: Kim J. Writing - original draft: Kim J. Writing - review & editing: Kim J, Cho E, Kim M, Kim J, Choo H, Lee JH.
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Abstract

Body weight (BW) is an economically important trait associated with meat production in the poultry industry. Despite their desirable meat quality, Korean native ducks (KNDs) exhibit lower BWs and slower growth rates than commercial breeds that limited their competitiveness. Due to the limited availability of duck-specific single-nucleotide polymorphism (SNP) chips and the high cost of large-scale genotyping, we previously developed a dual species SNP chip applicable to both ducks and chickens. In this study, we investigated the genetic variation of growth traits in KNDs and evaluated the applicability of the developed chip. BWs from hatching to 10 weeks of age were recorded and compared with those of Pekin ducks to assess growth performance. A genome-wide association study (GWAS) was conducted for body weight at 6 weeks (BW6) and at 8 weeks (BW8), corresponding to the commercially market age and represent commercial important time points, using 232 KNDs genotyped with 30K SNPs. This result confirmed that KNDs have relatively slow growth compared to Pekin ducks. GWAS identified a significant SNP associated with BW6, and four candidate genes (*ENSA*, *IQGAP3*, *ANP32E*, and *RAB25*) were located within the genomic region surrounding the significant SNP. However, no significant SNPs were detected for BW8. These outcomes may provide insights into the genetic basis of body weight traits in ducks and offer valuable information for the genomic breeding strategies in KNDs.

Keywords: Korean Native Ducks; Body Weight; Genetic Improvement; Genome-Wide Association Study; Candidate Genes; Single Nucleotide Polymorphism

Introduction

As the global population continues to grow, ensuring an adequate food supply and meeting nutritional demands have become critical issues in the coming decades. Consequently, improving production efficiency has become an increasingly important priority for animal breeders. In the world, particularly in Asia, ducks are emerging as an increasingly important source of meat. Ducks play a growing role in meeting nutritional demands [1, 2, 3]. Although poultry meat consumption continues to rise in Korea, approximately

90% of ducks raised for commercial purposes originate from foreign breeds, such as Cherry Valley (UK) and Grimaud (France). In contrast, Korean native ducks (KNDs) account for less than 10% of the total domestic duck population in Korea [4].

KNDs are renowned for their unique flavor and superior meat quality [5]. However, owing to their slower growth rates and smaller body sizes than those of imported commercial breeds, they represent a smaller proportion of the duck industry [4, 6]. To achieve sustainable food production and preserve genetic diversity, it is essential to comprehensively understand the genetic determinants of body weight and environmental influences in KNDs [7]. However, despite the importance of improving body weight traits, genetic studies in ducks, particularly in KNDs, remain limited due to high cost of genotyping platforms and challenges associated with large sample sizes. To address these limitations, we previously developed a dual-species SNP chip and performed a basic quality control (QC) following standard protocols [7]. However, its practical applicability in real genetic analyses has not yet been evaluated. Therefore, it is necessary to assess its usability in genetic studies such as genome-wide association studies (GWAS).

In the livestock industry, GWAS are widely used to identify genetic markers to identify genetic markers associated with economically important traits, including weight, disease resistance, and productivity. GWAS have been widely used to identify candidate genes associated with body weight and growth rate in ducks [1, 9, 10, 11]. In Sichuan shelduck breeds, *PCSK6*, *TOX2*, and *TOMM7* have been identified as candidate genes associated with growth rate [1]. Whole-genome analyses of Zhongshan ducks and other Chinese indigenous breeds, including the Jinding duck, Shan Partridge duck, and Liancheng white duck, have revealed *SEMA5B* and *MIB1* as growth-associated candidate genes [12]. Zhu et al. [13] also identified *EIF2AK3* as a gene associated with body weight in several Chinese breeds, including Shanma, Jinding, Gaoyou, Maple Leaf, and Shaoxing ducks. Among commercial breeds, Pekin ducks are one of the most widely used duck breeds worldwide. According to Zhou et al. [14], a mutation leads to the expression of *IGF2BP1*, resulting in a 15% increase in the body size of Pekin ducks. Recently, Yu et al. [15] identified *XKR4* and its haplotypes as candidate loci associated with body size in Pekin ducks using GWAS. However, no study has identified candidate genes associated with body weight or analyzed the functions of these genes in KNDs.

In this study, we performed a GWAS in a KND population to identify growth-related markers for improving the growth performance of KNDs and other indigenous breeds. In addition, we evaluated the practical applicability of the developed SNP chip. This method allowed us to identify candidate genes and loci associated with body weight. The results of this study are expected to advance our understanding of growth-related genetic architecture in KNDs and support the application of the developed SNP chip in future studies.

Materials and Methods

Ethics statement

All animal growth, care, and experimental procedures were conducted in accordance with the relevant guidelines and under consistent conditions. These procedures were approved by the Animal Care and Use Committee of the Poultry Research Center, National Institute of Animal Science (NIAS), Republic of Korea (NIAS2023-0617).

Animals

The KNDs utilized in this study originated from the hybridization between mallard ducks and indigenous Pekin ducks. KNDs exhibit two types of plumage colors, namely color and white [16, 17]. The NIAS established a consistent color line derived from native ducks collected from private farms. In addition, individuals exhibiting white plumage were selectively bred to develop a reliable white line. Each line was raised separately. A total of randomly selected from KND population consisting of 116 colored and 116 white ducks. To ensure balanced representation, an equal number of individuals were selected from each line. The sex distribution consisted of 52 males and 180 females.

Phenotype data processing and comparison

Body weight measurements of KNDs were recorded from hatching (BWH) to 10 weeks of age (BW10) at two-week intervals. A total of 232 individuals were initially included for body weight trait analysis. Quality

control procedures were applied to ensure the reliability of the phenotype data. Outlier detection was performed using Z-score thresholds. Values deviating by more than ± 3 standard deviations (SD) from the mean were excluded. No individuals were excluded during the quality control process, and all 232 individuals were retained for downstream analyses. To determine the significance of BW differences between datasets, Welch's t-test [18] was performed for each for each week, sex, color, and breed. This test was used to account for potential unequal variances and sample sizes between groups. Statistical significance was set at p-value < 0.05.

Genotype quality control and principal component analysis (PCA)

Genotyping was conducted using a dual-species 60K SNP chip [7, 19], resulting in an initial dataset comprising 66,025 SNPs. From this dataset, 33,933 SNPs corresponding to ducks were extracted based on chromosome and position information. Quality control was implemented with PLINK v1.9 to exclude SNPs with a genotype call rate below 90%, a minor allele frequency (MAF) less than 0.1, and a Hardy-Weinberg equilibrium (HWE) p-value below 1×10^{-6}. Consequently, 591 SNPs were excluded due to excessive missingness, 898 due to low MAF, and 1,378 due to deviation from HWE. After quality control, 32,342 high-quality SNPs across 232 samples were used in this study.

Principal component analysis (PCA) was performed on the filtered SNP dataset comprising 232 high-quality samples using PLINK v1.9 to assess population structure. PC1 and PC2 were incorporated as covariates in the genome-wide association analysis to account for potential population stratifications.

Genome-wide association analysis

GWAS for BW6, BW8 traits was performed using the genome-wide efficient mixed model association (GEMMA) software [20]. The linear mixed model (LMM) used in this study was defined as follows:

$$\boldsymbol{y} = \boldsymbol{W\alpha} + \boldsymbol{x\beta} + \boldsymbol{u} + \boldsymbol{e},$$

$\boldsymbol{y}$ is the vector of phenotypic values, $\boldsymbol{W}$ is the matrix of covariates with fixed effects (sex, hatching date, PC1, and PC2), and α is the corresponding effect size vector. Where x represents the vector of SNP genotypes, and $\boldsymbol{\beta}$ is the effect size of the SNP being tested. $\boldsymbol{u}$ is the random polygenic effect, assumed to

follow a normal distribution, $u \sim N(0, \sigma^2_g K)$, where $\boldsymbol{K}$ is the genetic relatedness matrix. $\boldsymbol{e}$ represents the residual errors, modeled as $e \sim N(0, \sigma^2_e I)$ [8]. To control for false positives due to multiple testing, a genome-wide significance threshold was set using the Bonferroni correction, calculated as 0.05 divided by the total number of SNPs [8].

The heritability of body weight trait was estimated using restricted maximum likelihood (REML) method in genome-wide complex trait analysis (GCTA) software [19].

Definition of candidate genomic regions based on GWAS results

We defined a significantly associated genomic region covering 1 Mb, centered on the most significant SNPs identified through GWAS, using the reference duck genome annotation (ZJU1.0, GCF_015476345.1) obtained from the NCBI database. To visualize the region around the lead SNPs, SNPs within ± 500 kb of each lead SNP were extracted from the GWAS results. Pairwise linkage disequilibrium (LD; r^2) values were calculated and merged with these SNPs, with colors assigned according to LD strength. The MAF for each SNP was calculated as the smaller value between allele frequency and its complement (1 - allele frequency).

Functional annotation analysis

Functional annotation was performed using g: Profiler [22] based on *Anas platyrhynchos* as the reference genome. The analysis included Gene Ontology (GO) enrichment of the significant SNPs and associated candidate genes identified in this study.

Results

Body weight of Korean native ducks

BW data were recorded for 232 KND samples. The population was categorized by strain (colored and white) and sex (male and female). Each strain consisted of 116 samples, including 32 males and 84 females in the colored strain, and 20 males and 96 females from the white strain. The descriptive statistics for BW from

hatching to 10 weeks of age are presented in Table 1 and Figure 1. Differences in BW across strains and sexes were assessed using Welch's t-test [18].

Across all groups, mean BW steadily increased with age. At hatch, BW ranged from 45.41 ± 5.24 g (colored males) to 47.40 ± 4.75 g (white males), with no significant differences were observed between strains or sexes (p-value = 0.5078). No sex-based differences were observed from hatching to 4 weeks of age. At 4 weeks (BW4), males (1,613.63 ± 140.86 g) were heavier than females (1,539.25 ± 168.19 g) (p-value = 0.0018). At 6 to 8 weeks, no significant differences were observed between sexes (BW6: p-value = 0.4703, BW8: p-value = 0.7873).

However, by BW10, sexual dimorphism became evident in both strains. Male BW reached 3,222.67 ± 275.04 g in the colored strain and 3,192.00 ± 582.31 g in the white strain, which were considerably higher than those of the corresponding female groups (2,860.00 ± 309.32 g and 2,843.88 ± 380.75 g) (p-value = 3.241×10^{-7}).

In the strain-based comparison, significant differences between colored and white strains were observed at BW2 and BW4 (BW2: p-value = 2.441×10^{-7}, BW4: p-value = 4.837×10^{-4}). Notably, in females, the colored strain consistently exhibited higher BW than the white strain from 2 to 10 weeks. As age increased, the SD of BW also increased, indicating that individual differences in growth became more evident.

Comparison of KNDs and Pekin ducks

To further compare growth patterns between breeds, the same statistical method Welch's t-test was applied to both KND and Pekin datasets [18]. For comparison, published data for Pekin ducks were referenced from Hasan et al. [23], who reported the mean ± standard error of weekly body weights for 219 individuals (109 females and 110 males).

At BWH, Pekin females and males were weighed 50.58 ± 3.76 g and 51.52 ± 3.78 g, while KND females and males weighed 46.71 ± 5.32 g and 46.17 ± 5.11 g, respectively. Pekin ducks were significantly heavier by approximately 5 g at this stage, as supported by the p-values (Female: p-value = 4.769×10^{-12}, Male: p-value = 2.524×10^{-9}). At BW2, Pekin females and males weighed 363.90 ± 74.96 g and 372.60 ± 74.26 g, whereas KND females and males weighed 604.31 ± 96.43 g and 591.27 ± 95.09 g. At BW2, KNDs exhibited

higher body weights than Pekin ducks (Female and Male: p-value $< 2.2 \times 10^{-16}$). By BW4, the average BWs became comparable between two breeds. Pekin ducks showed mean body weights of 1496.45 ± 174.04 g in females and 1,604.41 ± 200.22 g in males, while KNDs exhibited similar values 1,539.25 ± 168.19 g for females and 1,613.63 ± 140.86 g for males. At this stage, no significant difference was observed on males (p-value = 0.7362) although a slight difference remained in females (p-value = 0.0413).

However, from BW6, substantial differences emerged between breeds. At BW6, females and males of Pekin ducks reached at 2,809.93 ± 271.45 g and 3,104.34 ± 319.78 g, while KNDs reached females 1,993.72 ± 257.29 g and 1,971.35 ± 174.58 that Pekin ducks are heavier than KNDs (Female and Male: p-value $< 2.2 \times 10^{-16}$). This divergence became even more pronounced at BW8, which corresponds to the typical market age. Pekin females and males weighed 3,424.99 ± 298.28 g and 4,001.58 ± 399.18 g, while KND females and males weighed 2,620.36 ± 309.45 g and 2,632.12 ± 265.60 g, respectively. Pekin ducks were approximately 804.63 g heavier in females and 1,369.46 g heavier in males (Female and Male: p-value = 2.2×10^{-16}). As a result, KNDs were considerably lighter than Pekin ducks at market age.

Based on these findings, BW6 and BW8 were identified as important time points that growth differentiation was most pronounced. Therefore, we conducted GWAS using BW6 and BW8 to identify candidate genes associated with growth traits in KNDs. These results will provide genomic information that may support future breeding strategies for the KND breed.

Principal component analysis

We performed PCA prior to the GWAS to account for potential population stratification. The analysis identified two distinct genetic clusters among individuals. As presented in Figure 2, these clusters are illustrated by color (red) and white (blue) lines of KNDs. The KND population was divided into two groups (white line and colored line). NIAS developed the white line through selective breeding from colored lines to overcome difficulties associated with feather removal during processing for duck meat production [18]. These results also indicate that the developed SNP chip can detect the population structure, demonstrating its applicability in genetic analyses.

Genome-wide association study

In this study, BW6 and BW8 were selected as target traits to identify candidate genes associated with growth in KNDs, as these time points correspond to commercial market weights. The distributions of BW6 and BW8 in the final population after QC are presented in Figure 3. The estimated heritability of body weight was 0.017 for BW6 and 0.088 for BW8. GWAS was performed using 232 KNDs with 32,342 SNPs for BW6 and BW8. Based on the GWAS results and Bonferroni correction, one SNP located on chromosome 26 was significantly associated with BW6 (NC_051797.1:39142 T/C) (p-value = 5.45×10^{-7}) (Figure 4). This significant SNP was located near the proximal end of chromosome 26 within an intergenic region and did not overlap with any currently annotated genes. To elucidate the genomic context of the identified SNP, an initial window of 1Mb (±500 kb) centered on the lead SNP was considered. Within this region, LD analysis was performed using PLINK v1.9 to refine the candidate interval. However, no SNPs represented strong LD ($r^2 \geq 0.6$) with the lead SNP (Figure 5). This result indicates that the LD structure was not well-defined within this region. This result may be attributed to the limited genetic variability in the dataset, due to all individuals were originated from a single population within the same generation. Therefore, a physical distance (1Mb) was adopted. However, since the significant SNP was located near the beginning of the chromosome, the upstream region was physically constrained. As a result, a refined window of 550 kb (50 kb upstream and 500 kb downstream) was defined for further analysis. Within this defined region, a total of 46 genes were identified based on the ZJU1.0 genome annotation (Supplementary Table 1). Although relatively low LD was observed between the lead SNP and nearby variants (Figure 5), these genes were considered potential candidate genes based on their physical proximity to the associated locus.

The MAF of SNPs within the 550 kb region ranged from 0.1 to 0.5. The MAF of the lead SNP was approximately 0.25, indicating that this variant is relatively common in this population. This MAF enhances the reliability of this SNP as a candidate marker associated with body weight traits.

Among the identified candidate genes, the nearest gene to the significant SNP was identified as the Rh family B glycoprotein (*RHBG*) gene. The *RHBG* is located in the region of 23,303-28,643 bp on chromosome 26. Furthermore, LD analysis revealed that the second most significant SNP (NC_051797.1:45334), which exhibited the strongest LD (r^2 = 0.41) with the lead SNP among the

neighboring variants, is also located within an intergenic region (Figure 5). In summary, these results suggest that this locus represents a novel genomic region associated with duck growth traits that has not been previously reported. In contrast, no SNPs surpassed the genome-wide significance thresholds in the GWAS for BW8. Despite the limited significant findings, the GWAS demonstrated that the SNP chip can be applied in genetic research.

Functional annotation analysis

Functional annotation analysis was performed using g: Profiler [21] to investigate the biological significance of the candidate genes associated with duck body weight identified in the GWAS. Two GO molecular function terms were identified: phosphatase inhibitor activity (GO:0019212) (FDR = 0.0117) and myosin binding (GO:0017022) (FDR = 0.0043) (Table 2). Notably, both enriched terms are associated with pathways related to cell metabolism, cellular growth, and muscle development.

Discussion

In this study, KNDs exhibited lower body weights than Pekin ducks, particularly from 6 to 8 weeks of age (p-value = 2.2×10^{-16}), which corresponds to the commercial market period. Although KNDs showed slightly higher body weights from 2 weeks (p-value $< 2.2 \times 10^{-16}$), the difference became substantial after 6 weeks, with a clear divergence observed at 8 weeks. While Pekin ducks dominate the global market due to their rapid growth and heavier market weight, the relatively slower growth performance of KNDs limits their commercial competitiveness. Therefore, to overcome these limitations, it is essential to develop a new commercial strain that combines the rapid growth characteristics of Pekin ducks with the unique indigenous traits of KNDs. In this context, the identified candidate genes and loci in this study may provide valuable genomic information for breeding strategies in KNDs.

To establish a genetic foundation for developing an improved strain, it is crucial to understand the complex biological mechanisms regulating these growth traits. In this context, our functional annotation

analysis emphasizes the biological significance of identified GO terms with the candidate genes, offering valuable insights into the metabolism and signal networks governing duck growth.

Among the genes associated with phosphatase inhibitor activity (GO:0019212) (FDR = 0.0117), Endosulfine Alpha (*ENSA*) gene is known to inhibit protein phosphatase 2A (PP2A), a highly conserved serine/threonine phosphatase involved in multiple cellular processes [24]. This inhibition relies on phosphorylation at Ser67 by Greatwall kinase (Gwl), which acts as a regulator of PP2A activity during the cell cycle and metabolism.

PP2A plays an essential role in cellular signaling, including suppression of the mitogen-activated protein kinase (MAPK) pathway [25]. It is also noteworthy that PP2A functions as a negative regulator of insulin signaling by dephosphorylating and inactivating Akt and PKCλ. This inhibition reduces glucose transport, which is mediated through the Akt pathway [26]. Sailaja et al. [27] reported a significant association between an *ENSA* gene variant (rs1053732) and type 1 diabetes, suggesting that *ENSA* may influence insulin-related metabolic pathways. Based on these outcomes, genetic variation in *ENSA* may affect body weight by altering glucose homeostasis and insulin signaling pathways by regulating PP2A activity.

In addition to *ENSA*, Acidic Nuclear Phosphoprotein 32 family member E (*ANP32E*) gene is another phosphatase inhibitor involved in chromatin remodeling by eliminating histone variant H2A.Z. In brain tissue, *ANP32E*, along with Cpd1, regulates PP2A activity at synapses during synaptogenesis [28, 29]. Overexpression of *ANP32E* enhances the activation of the AKT/mTOR signaling pathway and promotes cellular metabolism, including cell growth and glycolysis [30-32]. Liu et al. [33] demonstrated the functional role of *ANP32E* in colorectal cancer cells using *ANP32E* knockdown models. According to their results, knockdown of *ANP32E* led to reduced AKT/mTOR signaling, decreased cell growth, and diminished glycolysis-related activity. These findings suggest that the *ANP32E* may regulate cell growth and metabolism through inhibition of PP2A activity.

Taken together, *ENSA* and *ANP32E* may represent key candidate genes associated with growth performance in KNDs, particularly during the market age stage, through their roles in metabolic regulation and cell growth pathways.

Within the set of genes associated with myosin binding (GO:0017022) (FDR = 0.0043), the IQ-motif-containing GTPase-activating protein 3 (*IQGAP3*) and Ras-related protein Rab-25 (*RAB25*) have been identified as candidate genes. Both genes are implicated in cellular signaling, cytoskeleton, and metabolism.

The IQ-motif-containing GTPase-activating protein (*IQGAP*) family encodes scaffold proteins that regulate cellular processes such as cell motility, intracellular signaling, and cytoskeletal structure. These functions are mediated through interactions with calcium-dependent proteins, particularly calmodulin (CaM) [34, 35]. *IQGAP3* gene, a member of this protein family, has been demonstrated to bind CaM [32], suggesting its involvement in calcium-mediated signaling cascades that regulate cellular processes and metabolism. Yang et al. [36] demonstrated that overexpressed *IQGAP3* enhances tumor cell proliferation, motility, and invasiveness. *IQGAP3* interacts with extracellular signal-regulated kinase 1 (ERK1), facilitating ERK1 phosphorylation upon epidermal growth factor (EGF) stimulation and thereby activating the ERK signaling pathway. The epidermal growth factor receptor (EGFR), a major growth factor receptor, initiates intracellular signaling cascades that promote cell proliferation. The EGFR-ERK signaling pathway is a crucial signal transmission pathway that transmits proliferative signals from the plasma membrane to the nucleus. *IQGAP3* promotes cell proliferation through Ras-dependent ERK activation [32]. These outcomes suggest that *IQGAP3* may contribute to body weight variation with regulation of cell signaling and structure in muscle tissue.

Overall, *IQGAP3* and *RAB25* genes may influence growth performance through their roles in cytoskeletal organization, intracellular signaling, and muscle development, which are critical for tissue growth during market age stages of KNDs.

Members of the RAS oncogene family, including RAB proteins, encode small GTPases that participate in diverse intracellular processes. These GTPases act as molecular switches and regulate essential cellular functions, including proliferation, differentiation, and survival, as a result of alternating between an active GTP-bound state and an inactive GDP-bound state [38, 39]. *RAB25* gene, a member of the Rab 11 subfamily, has been reported to regulate apical transport and recycling of vesicles, and functions either as an oncogene or a tumor suppressor in cancer cells [40-43]. According to Bannister et al. [44], *RAB25* is present in cerebral artery smooth muscle cells (myocytes), and it regulates the expression of CaV1.2 calcium channels.

Based on the role of CaV1.2 in gene expression and muscle contraction, essential for organ perfusion and blood pressure maintenance, *RAB25* may also contribute to skeletal muscle metabolism and development. Cheng et al. [45] demonstrated that *RAB25* enhances glucose uptake by activating the AKT signaling pathway. This activation facilitates the translocation of glucose transporters to the plasma membrane in cancer cells, promoting cell proliferation and survival under metabolically stressful conditions. These outcomes suggest that *RAB25* may influence body weight and muscle growth by modulating intracellular trafficking, calcium signaling, and glucose metabolism.

According to GO terms, candidate genes identified from GWAS results were associated with metabolic regulation and muscle development. These findings suggest that body weight trait in ducks is regulated by diverse networks involving metabolism and muscle-growth pathways, rather than by a few individual genes. This knowledge may serve as a foundation for breeding and selection programs in both indigenous and commercial duck breeds to improve body weight. Therefore, further validation and research in the future are necessary to clarify how specific genomic variations influence the body weight in ducks.

KNDs represent an important indigenous genetic resource. However, their phenotypic and genotypic characteristics remain poorly characterized. In this study, we compared live body weight with Pekin ducks and performed a GWAS to identify genetic loci associated with body weight in the KND population. The estimated heritability of body weight in this population was relatively low, 0.017 for BW6 and 0.088 for BW8. These traits are inherently complex and polygenic in nature, and their expression is known to be substantially influenced by non-genetic factors such as nutritional status, environmental conditions, and management practices [8]. A recent meta-analysis by Hossein-Zadeh [46], which reviewed 31 studies published between 1988 and 2024, reported mean heritability estimates of 0.396 for body weight and 0.154 for growth rate, further emphasizing the considerable influence of environmental variation on these traits.

However, to better understand this observation, we further examined the data structure. Although the use of low-density SNP (30K markers) and a relatively limited sample size (232 individuals) in this study may have contributed to reduced statistical power, additional investigation revealed that all samples were derived from single population within the same generation. As a result, the genomic relationship matrix exhibited low variance, indicating limited variability in genetic relationships among individuals. The lack

of variability restricted capturing the genetic structure and partition additive genetic variance, thereby contribute to the low heritability estimates observed in this study.

Previous large-scale GWAS in ducks even those using high-density genotyping platforms and relatively large sample sizes, have reported a limited number of significant loci associated with growth and body weight-related traits. For instance, Zhu et al. [9] identified four suggestive significant QTLs associated with body weight at body weight at 3 weeks using genotyping-by-sequencing in 639 Pekin ducks. These loci included three candidate genes *SLC10A2, SLC39A10, DNAJC19* with suggestive levels of association (*p*-value = 3.48×10^{-5}), located on chromosome 1, 7, and 9. Similarly, Liu et al. [10] identified eight significant SNPs associated with BWH and BW8 using whole-genome re-sequencing data in 199 Loumen ducks, with candidate genes distributed across chromosome 1, 2, 4, 7, and 11. Even with extensive genotyping and large sample sizes, only a few SNPs have been identified. These outcomes support the hypothesis that body weight and growth rate in ducks are governed by numerous small-effect loci and are substantially influenced by environmental factors. Consequently, identifying major QTL remains a significant challenge, despite the use of high-quality genomic datasets and large sample sizes.

Based on these results, the identified SNP (NC_051797.1:39142 T/C) and its flanking region represent a putative candidate region associated with growth traits in KNDs. However, this interpretation should be treated with caution. The effect allele (T) was associated with an increase body weight (β = 133.55 $\pm$ 25.89 g), as estimated using GEMMA. The genotype distribution of this SNP in the study population was n = 127 (C/C), n = 93 (C/T), and n = 12 (T/T).

One of the primary limitations of this study is the relatively small sample size (n = 232) and number of SNPs (32,342), which reduces the statistical power to detect true associations. The heritability (h^2 = 0.017 $\pm$0.12 for BW6 , and h^2 = 0.08 $\pm$0.17 for BW8 accompanied by large standard errors, indicating limited precision and suggesting that only a small proportion of phenotypic variance is explained by the SNPs included in this study. This result may be attributed to the limited sample size, as well as the reduced genetic variability resulting from the use of individuals derived from a single population within the same generation, which can lead to a poorly estimated genomic relationship matrix. Furthermore, no SNPs surpassed the

genome-wide significant thresholds for BW8. Overall, given these limitations, the identified locus should be considered as a putative candidate region rather than a locus for growth traits.

Despite these limitations, the developed SNP chip generated genotype data suitable for downstream analyses, including PCA and GWAS. Clear population structure was observed in PCA, and GWAS detected putative trait-associated loci, suggesting that the SNP chip can be effectively applied in genetic studies. However, the relatively small sample sizes (n = 232) limited the statistical power and reliability of these findings. Nevertheless, future studies with larger sample sizes are required to validate these results and further assess the performance of the SNP chip.

Conclusion

This study confirmed that KNDs exhibit lower body weights than Pekin ducks at market age (BW 6 to BW 8). To identify genetic factors associated with growth performance in KNDs, we applied a SNP chip to perform GWAS and identified a significant SNP in BW6 result. Within the associated region, *IQGAP3*, *ANP32E*, *ENSA*, and *RAB25* were suggested as candidate genes. These findings offer insights into the complex genetic basis of growth-related traits in KNDs and emphasize potential genomic regions for further investigation. This study demonstrated that the developed SNP chip can be effectively applied in GWAS. The identified genes and loci may serve as molecular markers for marker-assisted selection and genomic breeding strategies, ultimately facilitating genetic improvement in KND populations. However, due to the limited sample size and population structure of this study, further research with large datasets and more comprehensive phenotype data is necessary to increase the accuracy and robustness of the results.

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Availability of data and material

The genotype data of this study are available from Figshare at a private link for review purposes (https://figshare.com/s/ddb9211f89c13d1f2b0d). The data will be made publicly accessible upon publication of the article.

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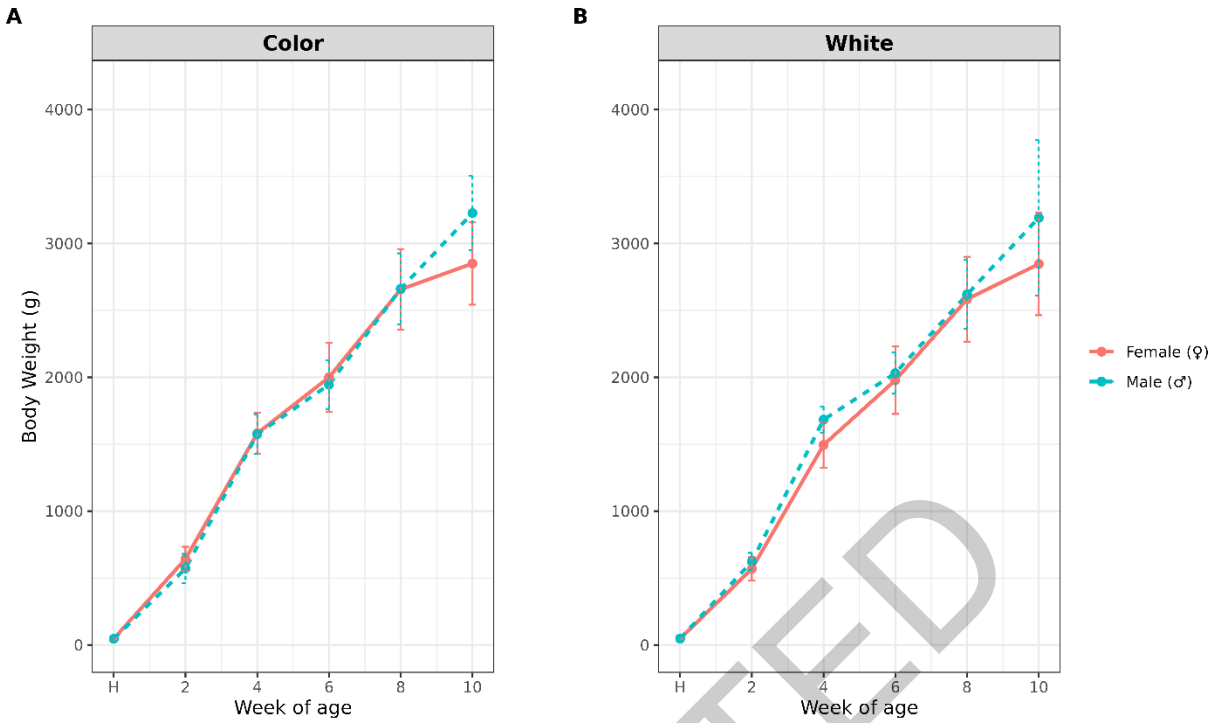
Tables and Figures

Table 1. Body weight (mean ± SD, g) and coefficient of variation (CV, %) of color and white strains by sex from hatch to 10 weeks of age

Strain	Sex	BWH (g) / CV (%)	BW2 (g) / CV (%)	BW4 (g) / CV (%)	BW6 (g) / CV (%)	BW8 (g) / CV (%)	BW10 (g) / CV (%)	Num
Color	Male	45.4 ± 5.24 (11.54)	571.34 ± 106.19 (18.59)	1,568.55 ± 147.89 (9.43)	1,934.22 ± 178.75 (9.24)	2,639.69 ± 273.82 (10.37)	3,222.67 ± 275.04 (8.53)	32
	Female	46.5 ± 5.50 (11.93)	642.76 ± 89.40 (13.91)	1,585.36 ± 153.67 (9.69)	2,011.90 ± 266.69 (13.26)	2,665.60 ± 299.58 (11.24)	2,860.00 ± 309.32 (10.82)	84
White	Male	47.4 ± 4.75 (10.02)	623.15 ± 64.27 (10.31)	1,683.50 ± 96.06 (5.71)	2,030.75 ± 153.75 (7.57)	2,620.00 ± 258.41 (9.86)	3,192.00 ± 582.31 (18.24)	20
	Female	46.8 ± 5.14 (10.96)	570.78 ± 89.97 (15.76)	1,499.38 ± 170.75 (11.39)	1,977.81 ± 249.09 (12.59)	2,580.78 ± 314.05 (12.17)	2,843.88 ± 380.75 (13.39)	96
Total	Male	46.17 ± 5.11 (11.06)	591.27 ± 95.09 (16.08)	1,613.63 ± 140.86 (8.73)	1,971.35 ± 174.58 (8.86)	2,632.12 ± 265.60 (10.09)	3,210.40 ± 420.10 (13.09)	52
	Female	46.71± 5.32 (11.39)	604.31 ± 96.43 (15.96)	1,539.25 ± 168.19 (10.93)	1,993.72 ± 257.29 (12.91)	2,620.36 ± 309.45 (11.81)	2,851.49 ± 347.99 (12.20)	180

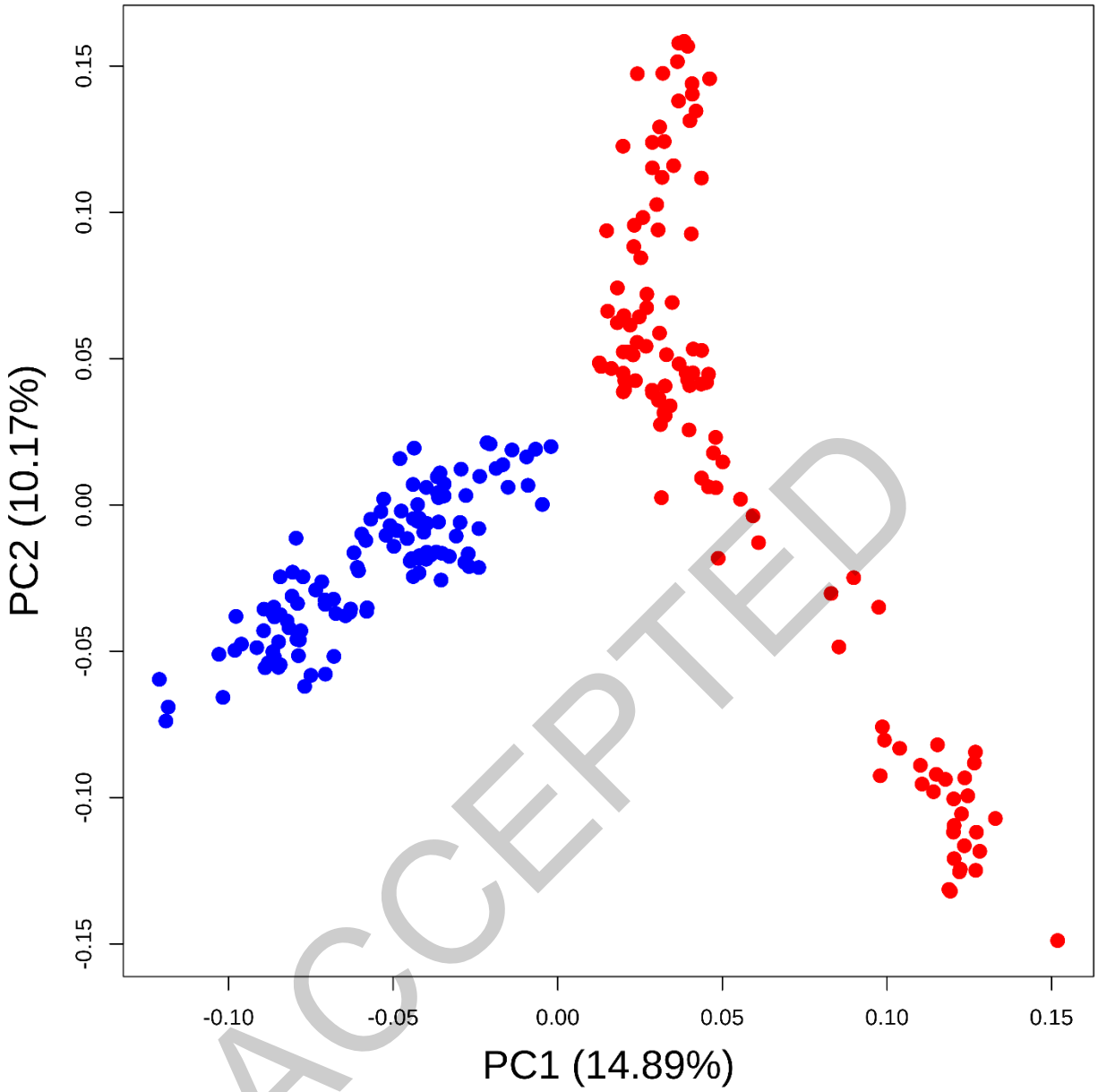
SD, standard deviation; CV, coefficient variation; BW, body weight; H, hatch; BWH, body weight at hatch; BW2, body weight at 2 weeks; BW2, Body weight at 4 weeks; BW6, Body weight at 6 weeks; BW8, Body weight at 8 weeks; BW10, Body weight at 10 weeks; Num, number.



510

511 **Figure 1.** Growth curves of body weight for color and white strains from hatch to 10 weeks of age. Blue
512 color represents males, and red color represents females. (A) Growth curve of body weight for color strains.
513 (B) Growth curve of body weight for white strains. Dots represent mean body weights, and error bars
514 represent standard deviations.

515

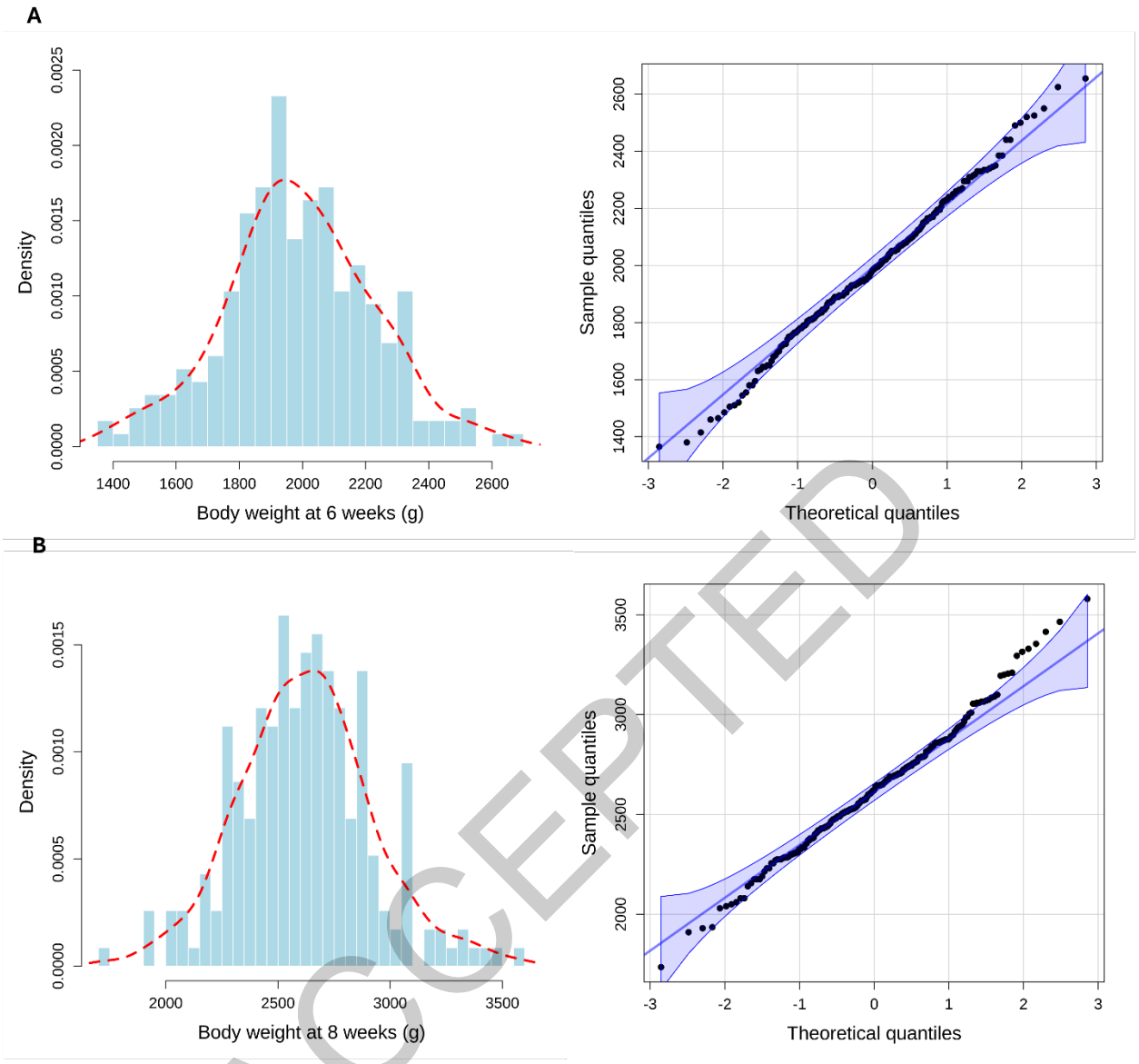


516

517 **Figure 2.** Principal component analysis of Korean native ducks analyzed in this study. Individuals were

518 grouped based on strain information into two groups: color strain (red) and the white strain (blue).

519

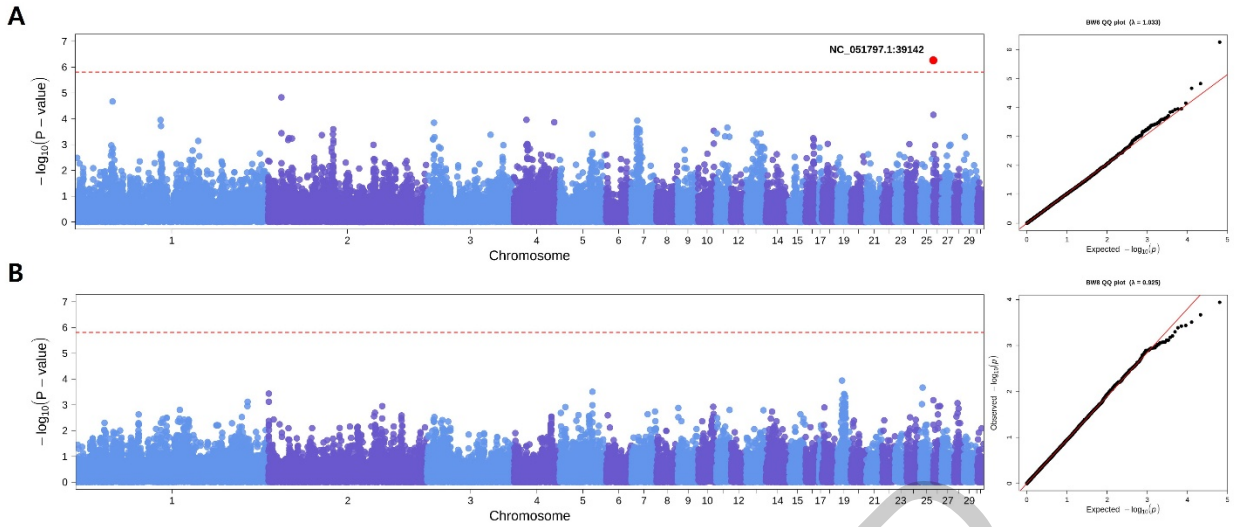


520

521 **Figure 3.** Distribution of body weight and growth rate traits. (A) Histogram and quantile-quantile plots for

522 body weight at 6 weeks. (B) Histogram and quantile-quantile plots for body weight at 8 weeks.

523



524

525 **Figure 4.** Manhattan plot for body weight and growth rate traits in Korean native ducks. The horizontal red
526 line indicates the genome-wide significance threshold, calculated using Bonferroni correction (*p*-value =
527 0.05 / total number of single nucleotide polymorphisms (SNPs)). (A) Manhattan plot for body weight at 6
528 weeks. (B) Manhattan plot for body weight at 8 weeks. No genome-wide significant SNPs were detected
529 according to the Bonferroni-adjusted threshold.

530

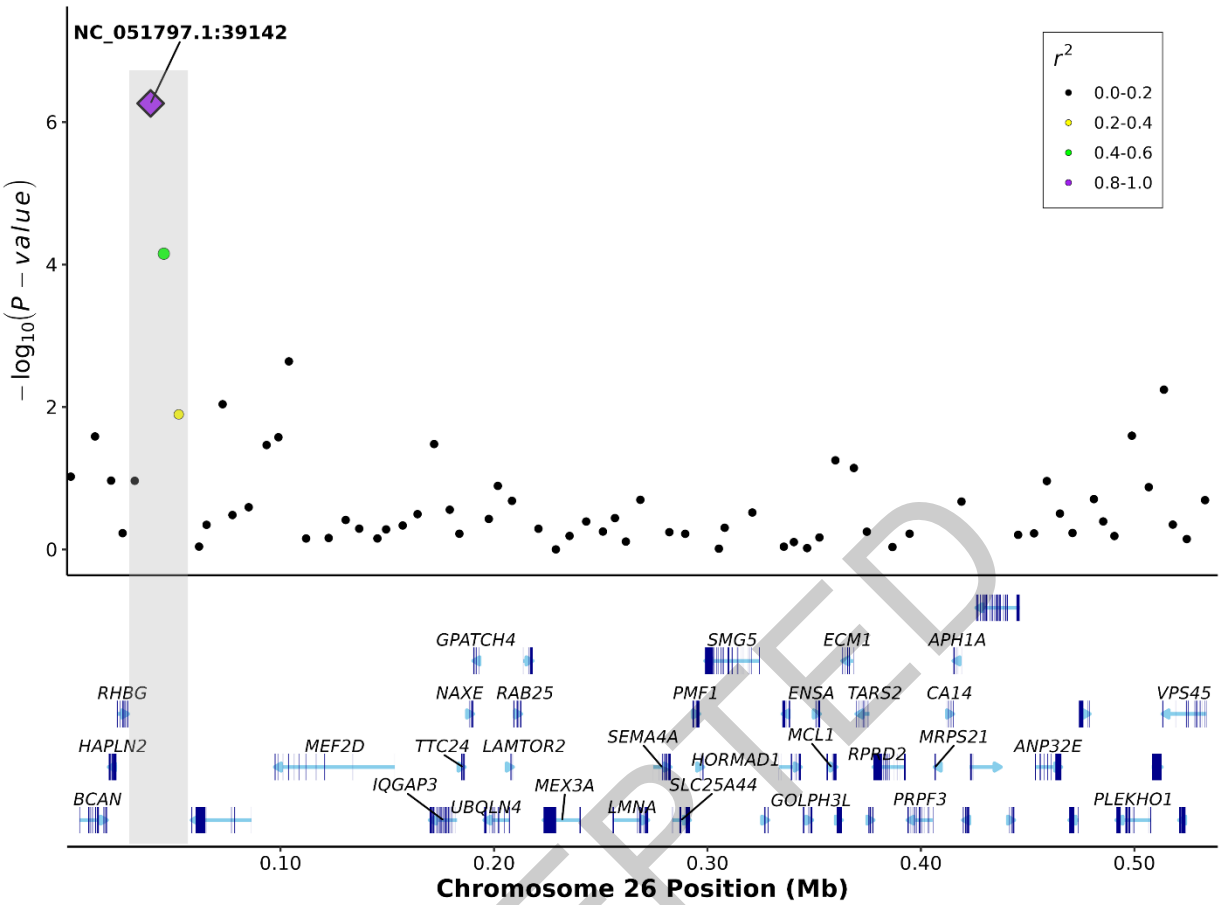


Figure 5. Regional association plot surrounding the significant single nucleotide polymorphism (SNP) (NC_051797.1:39142) associated with body weight at 6 weeks. The upper panel shows the -log$_{10}$ (*p*-value) of SNPs located within a window spanning 50 kb upstream to 500 kb downstream of the lead SNP. This window was selected to enhance visualization of local linkage disequilibrium (LD) structure and association signals, as SNP density was relatively low in the upstream region. Each SNP is color-coded based on its LD (r^2) with the lead SNP. The lower panel provides gene annotations, indicating the positions and structures of genes located within the genomic region. LOC genes are included but not labeled for clarity.

Table 2. Significant (*p*-value < 0.05) gene ontologies enriched for genes within 550kb flanking region of associated SNP for body weight trait

Category	GO ID	GO term	No. of genes	*p*-value	Gene names
Molecular function	GO:0019212	Phosphatase inhibitor activity	2	1.457×10^{-7}	*ENSA*, *ANP32E*
	GO:0017022	Myosin binding	2	3.607×10^{-3}	*IQGAP3*, *RAB25*

SNP, single nucleotide polymorphism; GO, Gene ontology.