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GENOME-WIDE ASSOCIATION ANALYSIS OF GROWTH AND DEVELOPMENT TRAITS IN QIRA BLACK SHEEP USING A 50K SNP CHIP ARRAY IN XINJIANG, CHINA

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ABSTRACT Growth and development traits in sheep are complex quantitative traits regulated by multiple genes with small effects. In this study, 186 Qira Black sheep were genotyped using the Illumina Ovine SNP50K BeadChip, and genome-wide association studies were performed for 11 growth-related traits using a mixed linear model. After quality control and association analysis, significant SNPs were identified and annotated to 104 candidate genes based on the Oar_v4.0 reference genome. Gene Ontology enrichment analysis indicated that these genes were mainly involved in skeletal system development, regulation of growth, cell proliferation, and developmental processes. KEGG pathway analysis revealed significant enrichment in growth hormone synthesis, secretion and action, insulin secretion, cGMP–PKG signaling pathway, and apelin signaling pathway. These pathways are closely associated with endocrine regulation and growth-related signaling mechanisms. The identified candidate genes provide valuable molecular resources for marker-assisted selection and genetic improvement of Qira Black sheep.

KEY WORDS Illumina Ovine SNP50K BeadChip, population structure, Qira Black sheep, growth, Plink, TASSEL

1. INTRODUCTION

Qira Black sheep (*Ovis aries*) are a native sheep breed in China, mainly distributed in the arid and semi-arid regions of southern Xinjiang. This breed is valued for its unique black wool color and good wool quality, making it an important local genetic resource. Understanding the genetic basis of economically important traits in Qira Black sheep is essential for sustainable breeding and genetic improvement. Recent genomic studies have begun to clarify the genetic architecture of key traits in this population. For example, Zhou *et al.* (2025) analyzed three types of wool color in Qira Black sheep and identified genes strongly associated with black wool production. These findings highlight the increasing application of genomic tools in breeding programs for this indigenous breed.

With the development of molecular breeding technologies, genetic improvement in livestock has accelerated significantly. Following the implementation of the HapMap Project and whole-genome sequencing initiatives, as well as the development of commercial high-density SNP chips for livestock species (Ragoussis *et al.,* 2009), genome-wide association studies (GWAS) have become a powerful approach for identifying candidate genes underlying complex quantitative traits. In sheep, GWAS has been widely applied to detect genomic regions associated with growth and body size traits, providing valuable insights into the molecular mechanisms influencing economically important characteristics (Li *et al.,* 2013). For instance, Li *et al.* (2024) identified significant SNPs and candidate genes associated with growth traits in Hu sheep using the Illumina Ovine SNP40K BeadChip. Similarly, Tao *et al.* (2021) analyzed 188 Qira Black sheep using a 630K SNP chip and detected candidate genes involved in muscle differentiation, metabolism, and cellular processes. Tuersuntuoheti *et al.* (2023) further confirmed that growth-related traits such as body height, body length, and chest girth are quantitative in nature and controlled by multiple genes.

In addition to growth traits, GWAS has also been used to investigate other economically important characteristics in sheep. Zhou *et al.* (2025) explored the genetic basis of the hornless (polled) trait in Qira Black sheep and identified loci associated with horn development. Bi *et al.* (2025) examined the effects of specific genes on seasonal reproductive traits in Qira Black sheep and related breeds. Moreover, Kim *et al.* (2016) identified genes associated with adaptation to hot arid environments, including those influencing thermo-tolerance, body size, and developmental traits in goats and sheep.

Although several studies have investigated growth-related traits in sheep, limited information is available regarding the genetic basis of growth and development traits in Qira Black sheep using medium-density SNP panels. Therefore, the present study aimed to perform a genome-wide association analysis of 11 growth and development traits in Qira Black sheep using the Illumina Ovine SNP50K BeadChip. Significant SNPs were annotated to candidate genes based on the Oar_v4.0 reference genome, and functional enrichment analyses using Gene Ontology (GO) and KEGG databases were conducted to explore the biological pathways underlying these traits. The findings of this study will contribute to a better understanding of the genetic mechanisms of growth traits and provide potential molecular markers for genetic improvement of Qira Black sheep.

2. MATERIALS AND METHODS

2.1. ANIMAL ETHICS

This study was reviewed and approved by the Science and Technology Ethics Committee of Tarim University (Approval No.: PA20260312003).

2.2 EXPERIMENTAL ANIMALS AND DNA EXTRACTION

A total of 186 healthy Qira Black sheep ewes aged 1–3 years were selected from Xinjiang Hetian Qira Jinken Agricultural and Animal Husbandry Science and Technology Co., Ltd., and maintained under uniform management conditions. Jugular vein blood samples were collected for genomic DNA extraction.

DNA was extracted using a FinePure Blood/Cell/Tissue Genomic DNA Extraction Kit (Jifan Biotechnology Co., Ltd., Beijing, China) following the manufacturer's protocol. DNA quality was evaluated by 1.5% agarose gel electrophoresis in 1× TBE buffer, and concentration was measured using a nucleic acid analyzer (Shenzhen Enke Biotechnology Co., Ltd., China). Qualified DNA samples were genotyped using the Illumina Ovine SNP50K BeadChip.

2.3 QUALITY CONTROL

Raw genotype data were processed using BeadScan and GenomeStudio (Illumina Inc., USA). Quality control was conducted using PLINK v1.9 (64-bit, 22 Oct 2024).

Samples with call rates <90% were excluded. SNPs were removed if they had (1) missing genotype rate >5%, (2) minor allele frequency (MAF) <0.05, or (3) Hardy–Weinberg equilibrium $P < 1\times10^{-6}$. After filtering, high-quality SNPs and individuals were retained for subsequent analyses.

2.4 POPULATION STRUCTURE ANALYSIS

Principal component analysis (PCA) was performed using PLINK v1.9 and R based on quality-controlled genotype data to evaluate population structure among the 186 animals.

2.5 GENOME-WIDE ASSOCIATION ANALYSIS

GWAS was conducted using TASSEL 5 under a mixed linear model (MLM) framework (Bradbury *et al.,* 2007). Genotype and phenotype datasets were merged to retain common individuals.

Eleven growth and development traits were analyzed: hip height (HH), hip width (HW), body height (BH), rump height (RH), body length (BL), chest girth (ChG), chest width (ChW), abdominal girth (AG), cannon bone circumference (CBC), tail length (TL), and tail width (TW).

After quality control, 48,935 SNPs were included in the analysis. The genome-wide significance threshold was determined using Bonferroni correction ($P < 0.05/48{,}935 = 1.02 \times 10^{-6}$), and the suggestive threshold was set at $P < 1/48{,}935$ (2.04×10^{-5}). Manhattan and Q–Q plots were generated in R.

2.6 CANDIDATE GENE ANNOTATION

As no SNP reached genome-wide significance, SNPs with $P < 1 \times 10^{-3}$ were selected for exploratory annotation. Their physical positions were mapped to the *Ovis aries* reference genome (Oar_v4.0). Genes located within ±50 kb of significant SNPs were considered candidate genes and used for downstream functional analysis. Genomic distributions were visualized using R.

2.7 FUNCTIONAL ENRICHMENT ANALYSIS

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using DAVID Bioinformatics Resources. GO categories included biological process (BP), cellular component (CC), and molecular function (MF).

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Significant enrichment was determined using a Benjamini–Hochberg false discovery rate (FDR) < 0.05. Enrichment results were visualized in R using the "ggplot2" package.

3. RESULTS

3.1. QUALITY CONTROL

The initial dataset consisted of 186 individuals and 53,093 SNP markers obtained from the Illumina Ovine SNP50K BeadChip. After applying the individual call rate threshold (<0.90), no samples were excluded, and all 186 individuals were retained for subsequent analyses.

SNP quality filtering was then performed sequentially. Filtering based on SNP call rate (<0.95) removed 165 SNPs, leaving 52,928 markers. Application of the minor allele frequency (MAF < 0.05) criterion eliminated 3,992 SNPs, resulting in 48,936 remaining variants. Finally, one SNP was excluded due to significant deviation from Hardy–Weinberg equilibrium (HWE, $P < 1 \times 10^{-6}$). After all quality control procedures, a total of 48,935 high-quality SNPs and 186 individuals were retained for subsequent genome-wide association analyses (Table 1).

Table 1. The result of quality control of SNP

QC Step	Before	After	Removed
Individuals (call rate < 0.9)	186	186	0
SNPs (call rate < 0.95)	53093	52928	165
SNPs (MAF < 0.05)	52928	48936	3992
SNPs (HWE p < 1e-6)	48936	48935	1

3.2. POPULATION STRUCTURE ANALYSIS

Principal component analysis (PCA) was conducted based on quality-controlled genotype data to evaluate the genetic structure of the 186 Qira Black sheep. The first two principal components (PC1 and PC2) are presented in Figure 1.

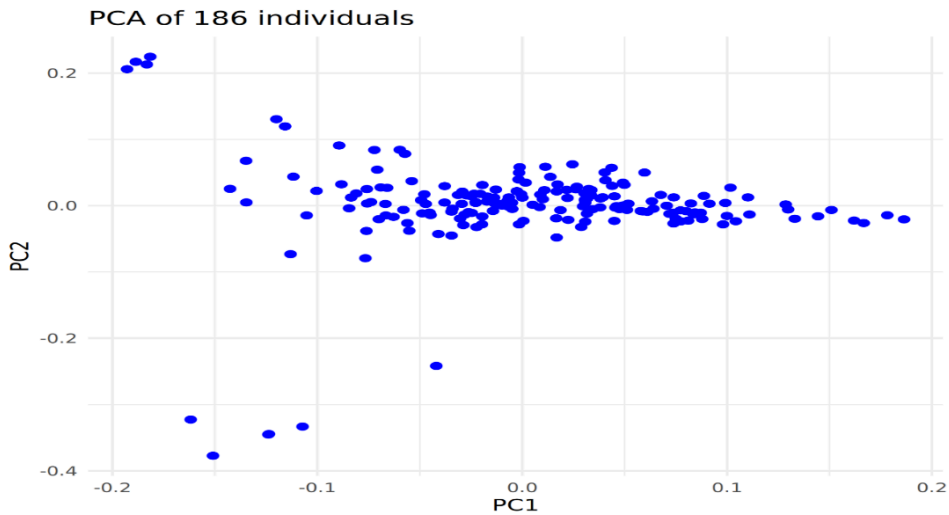


Figure 1. Principal component analysis (PCA) plot showing the genetic structure among 186 Xinjiang Qira Black sheep. The first two principal components (PC1 and PC2) indicate minimal

population stratification within the studied population.

The PCA plot revealed that the majority of individuals formed a primary cluster, indicating a relatively homogeneous genetic background within the sampled population. Most animals were distributed closely along the PC2 axis, suggesting limited genetic differentiation among individuals.

However, several individuals were separated from the main cluster along the PC1 axis, forming small distinct groups on the left side of the plot. In particular, a small cluster of individuals located in the upper-left quadrant showed tight grouping, which may reflect closer genetic relatedness within this subgroup. A few additional individuals were observed in the lower-left quadrant, indicating mild genetic divergence.

Overall, the PCA results suggest that the Qira Black sheep population exhibits a generally uniform genetic structure with limited substructure, supporting the suitability of this dataset for subsequent genome-wide association analysis.

3.3. GENOME WIDE ASSOCIATION STUDIES (GWAS)

Genome-wide association analyses were conducted for 11 growth and conformation traits—hip height (HH), hip width (HW), body height (BH), rump height (RH), body length (BL), chest girth (ChG), chest width (ChW), abdominal girth (AG), cannon bone circumference (CBC), tail length (TL), and tail width (TW)—in 186 Qira Black sheep genotyped using the Illumina Ovine SNP50K BeadChip. Manhattan and quantile–quantile (Q–Q) plots for each trait are presented in Figure 2(a–k) and Figure 3 (a-k).

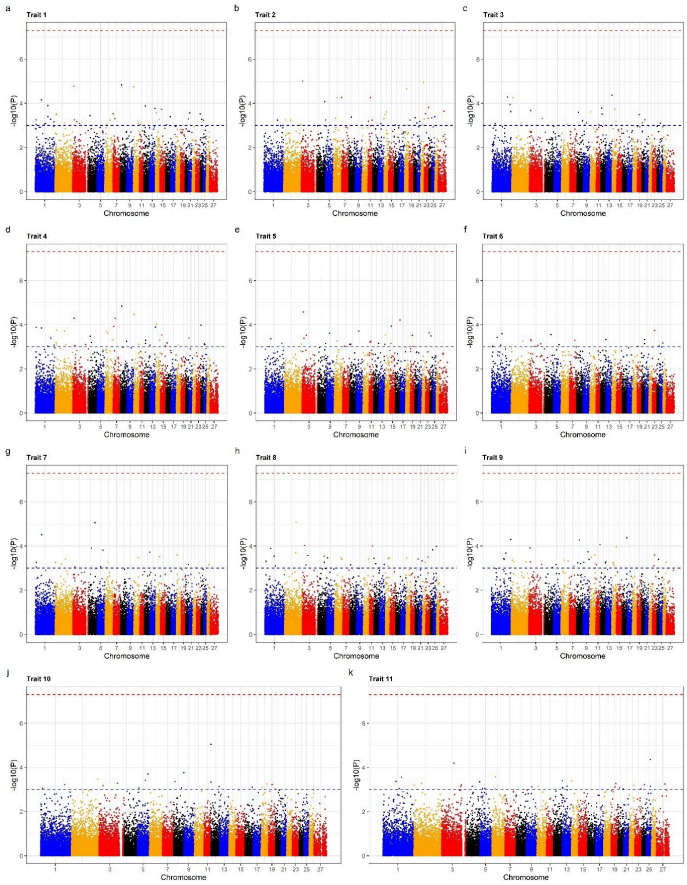


Figure 2. *Manhattan plots of genome-wide association analysis for 11 growth and development traits in Xinjiang Qira Black Sheep. (a) HH, hip height; (b) HW, hip width; (c) BH, body height; (d) RH, rump height; (e) BL, body*

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length; (f) ChG, chest girth; (g) ChW, chest width; (h) AG, abdominal girth; (i) CBC, cannon bone circumference; (j) TL, tail length; and (k) TW, tail width.

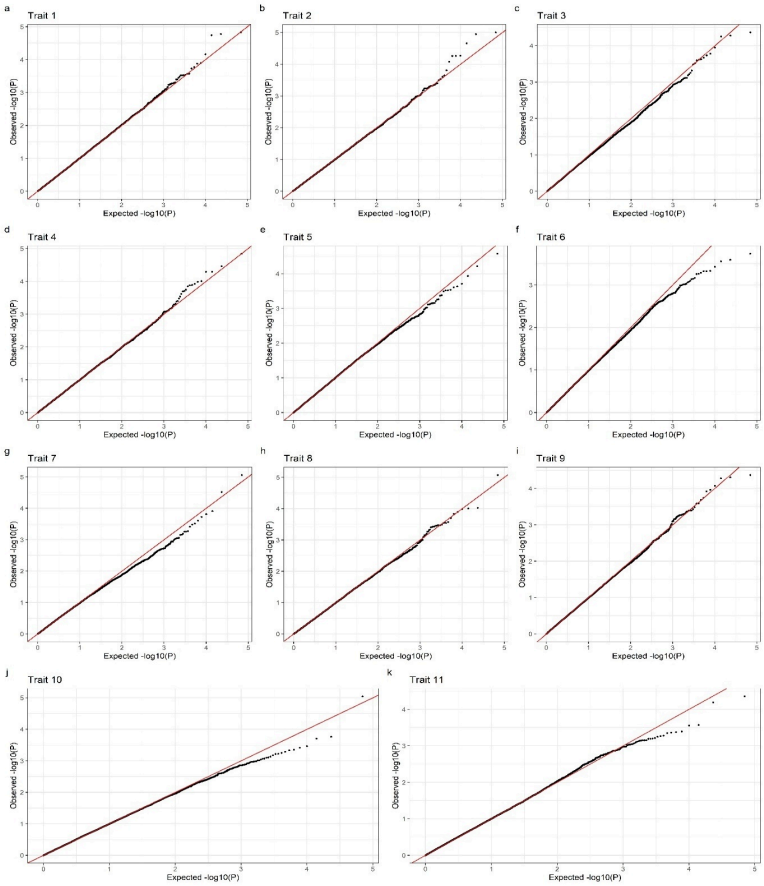


Figure 3. *Quantile–quantile (Q–Q) plots for the 11 analyzed traits showing the distribution of observed versus expected –log10(P) values. (a) HH, hip height; (b) HW, hip width; (c) BH, body height; (d) RH, rump height; (e) BL, body length; (f) ChG, chest girth; (g) ChW, chest width; (h) AG, abdominal girth; (i) CBC, cannon bone circumference; (j) TL, tail length; and (k) TW, tail width.*

Across traits, association signals were distributed across multiple chromosomes. For HH, the strongest signals were observed on chromosomes 3, 8, and 10 ($P = 1.48 \times 10^{-5}$ to 1.82×10^{-5}). HW exhibited notable loci on chromosomes 3, 18, and 22, with the most significant association detected on chromosome 3 ($P = 9.86 \times 10^{-6}$). BH showed association signals on chromosomes 1, 2, and 13 (P ranging from 4.28×10^{-5} to 5.54×10^{-5}), while RH demonstrated a peak on chromosome 8 ($P = 1.43 \times 10^{-5}$).

For BL, suggestive associations were identified on chromosomes 3 and 16 ($P = 2.65 \times 10^{-5}$ and 6.12×10^{-5}). ChG displayed a signal on chromosome 23 ($P = 1.83 \times 10^{-4}$). ChW and AG showed relatively strong signals on chromosomes 4 and 2 ($P = 8.72 \times 10^{-6}$ and 8.42×10^{-6}, respectively). CBC was associated with loci on chromosomes 1, 8, and 16 (P ranging from 4.27×10^{-5} to 5.26×10^{-5}). In addition, TL showed a signal on chromosome 12 ($P = 9.00 \times 10^{-6}$), and TW exhibited associations on chromosomes 3 and 25 ($P = 6.47 \times 10^{-5}$ and 4.41×10^{-5}, respectively).

Using the Bonferroni-corrected genome-wide significance threshold ($P < 1.02 \times 10^{-6}$), no SNP reached formal genome-wide significance. However, several loci exceeded the suggestive threshold ($P < 2.04 \times 10^{-5}$), particularly for HW (chromosome 3), ChW (chromosome 4), AG (chromosome 2), and TL (chromosome 12). These regions may

represent potential genomic loci associated with growth and conformation traits in Qira Black sheep and warrant further validation.

3.4. CANDIDATE GENES

Based on the nominal association threshold ($P < 1 \times 10^{-3}$), a total of 2,551 genes located within ±50 kb of associated SNPs was identified across the 11 growth-related traits. These genes were distributed across multiple chromosomes, consistent with the polygenic architecture of growth and body conformation traits.

Among these, several loci exhibited relatively stronger association signals ($P < 2.04 \times 10^{-5}$), including regions harboring *ROS1, VGLL2, KIDINS220, AHCYL1, OPTC, SH2D4B, CAPS2*, and others (Table 2). These genes are located in genomic regions that may contribute to variation in skeletal growth, cellular signaling, and developmental processes.

To further explore the biological relevance of the identified genes, Gene Ontology (GO) and KEGG pathway enrichment analyses were performed.

3.5. FUNCTIONAL ANALYSIS (GO AND KEGG)

To explore the biological relevance of the identified candidate genes, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using DAVID. A total of 2,551 candidate genes derived from SNPs with nominal association signals ($P < 1 \times 10^{-3}$) were included in the analysis.

GO enrichment analysis revealed significant overrepresentation of terms primarily related to developmental and regulatory processes. Among the Biological Process (BP) category, the most significantly enriched terms (FDR < 0.05) included regulation of transcription by RNA polymerase II (FDR = 1.15×10^{-5}), protein phosphorylation (FDR = 1.16×10^{-4}), and regulation of GTPase activity (FDR = 1.26×10^{-4}). These terms involve genes such as *MAPK1, MAPK3, PRKD1, PRKD2, SMAD4, RUNX1, GLI2, VGLL2,* and *NFIA*, which are known to participate in cell signaling, transcriptional regulation, and developmental processes (Fig. 4 Table 3).

The term activation of GTPase activity was also enriched (FDR = 2.89×10^{-2}), although with a relatively higher FDR value. Overall, the enrichment of transcriptional regulation and kinase activity-related processes supports the involvement of signaling networks in growth and body conformation traits.

KEGG pathway enrichment analysis further identified several significantly enriched pathways (FDR < 0.05). The top pathways included growth hormone synthesis, secretion and action, insulin secretion, apelin signaling pathway, vascular smooth muscle contraction, and cGMP–PKG signaling pathway (Fig. 5 Table 4). Additional enriched pathways related to endocrine regulation included GnRH secretion, cortisol synthesis and secretion, aldosterone synthesis and secretion, and oxytocin signaling pathway.

These enriched pathways are primarily associated with hormonal regulation, intracellular signaling, and metabolic control, which are biologically relevant to growth and development processes. Collectively, the enrichment results suggest that genes located near associated SNPs may participate in regulatory and signaling pathways influencing growth-related phenotypes in Qira Black sheep. However, further validation studies are required to confirm their functional roles.

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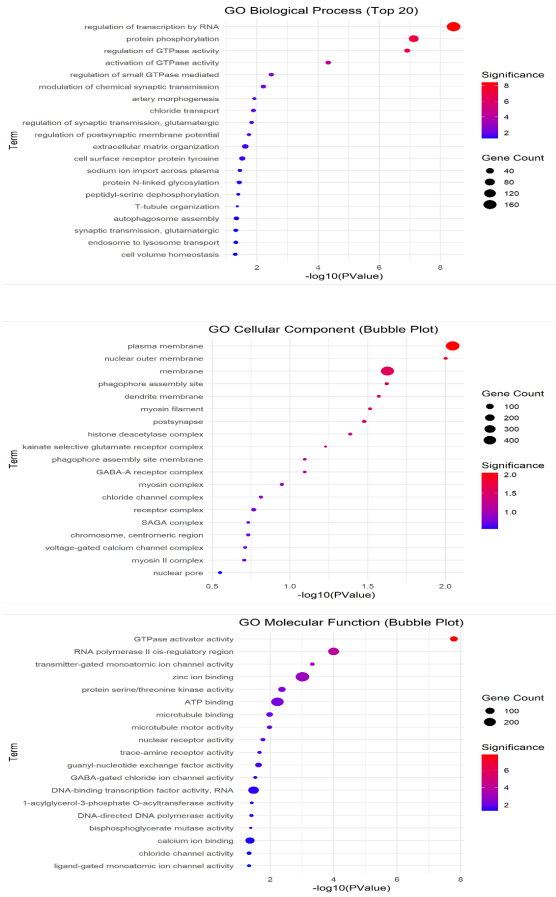


Figure 4. Gene Ontology (GO) enrichment analysis of candidate genes associated with growth traits. The top five significantly enriched terms from biological process (BP), molecular function (MF), and cellular component (CC) categories are shown.

4. DISCUSSION

Growth and developmental traits are primary determinants of meat production efficiency and economic performance in sheep. Numerous genome-wide association studies (GWAS) have demonstrated that growth traits are controlled by complex polygenic architectures. For example, Lu *et al.* (2020) identified 30 candidate genes, including *AADACL3, VGF, NPC1* and *SERPINA12,* associated with body weight in fine-wool sheep. Li *et al.* (2024) analyzed 567 Hu sheep and identified genes related to early weight, weaning weight (*FGF9, BMPR1B*), and monthly growth traits. Ghasemi *et al.* (2019) performed GWAS for birth weight in Lori-Bakhtiari sheep, and Kominakis *et al.* (2017) reported 39 genes associated with body size traits. Additionally, Jiang *et al.* (2021) identified *KITLG* and *CADM2* as candidate genes related to body height in Hu sheep. Together, these studies highlight the genetic complexity of growth-related traits in sheep.

In the present study, we conducted GWAS on 186 Qira Black sheep for eleven morphometric traits. By integrating association analysis with Gene Ontology (GO) and KEGG pathway enrichment, we identified candidate genes and biological pathways potentially underlying skeletal size, longitudinal growth, body width, body girth, and cannon bone circumference.

SKELETAL SIZE TRAITS (HH, BH, RH)

Skeletal dimensions such as hip height (HH), body height (BH), and rump height (RH) are key indicators of overall body structure and growth potential. Chromosome 8 harbored the strongest association signals, where *ROS1* and *VGLL2* were co-localized and significantly associated with both HH and RH, suggesting pleiotropic effects.

ROS1 encodes a receptor tyrosine kinase involved in protein phosphorylation and cell surface receptor signaling pathways regulating proliferation and development (Boulanger *et al.,* 2024). Although mainly studied in human oncology, these processes are fundamental to skeletal growth. *VGLL2* regulates transcription by RNA polymerase II and plays a role in muscle and tissue development (Honda *et al.,* 2024), suggesting coordinated regulation of musculoskeletal development.

Other associated genes included *BCAS1*, *SLC7A14*, *CLDN11*, and *SKIL*, while *SLC25A24* was detected for HH on chromosome 1. Both *SLC7A14* and *SLC25A24* belong to the solute carrier family, which mediates amino acid and mitochondrial metabolite transport. *SLC25A24* influences cell proliferation and apoptosis via mitochondrial signaling pathways (Yan *et al.,* 2025), indicating that intracellular energy balance may contribute to skeletal development.

Functional enrichment analysis identified genes involved in regulation of GTPase activity (*ARHGAP27, ARHGAP15, RASAL1, RASAL2*), transcriptional regulation (*NFKB1, GLI2, IRF6, CREB3L3, NFIA*), and protein phosphorylation (*RAF1, BRAF, MAP2K2, TTN*). *ARHGAP* genes regulate actin cytoskeleton remodeling and cell motility (Huang *et al.,* 2024; Gao *et al.,* 2024), processes essential for tissue morphogenesis. *GLI2*, a key transcription factor in the Hedgehog signaling pathway, regulates osteogenesis through *RUNX2* activation (Huang *et al.,* 2024), while *NFIA* modulates cellular differentiation and tissue development (Wu *et al.,* 2025).

The enrichment of *RAF1* and *MAP2K2* in growth hormone (GH), GnRH, apelin, and oxytocin signaling pathways supports the role of endocrine-regulated MAPK/ERK signaling in skeletal growth. GH has been shown to significantly affect body height, hip height, and chest depth (Wu *et al.,* 2020), and MAP family genes such as *MAP3K1* have been associated with body weight traits (Xiang *et al.,* 2024). Furthermore, *CREB3L3* was enriched in GH synthesis and action, insulin secretion, cGMP–PKG signaling, and aldosterone pathways. Previous studies demonstrated that *CREB3L3* regulates body weight, lipid metabolism, and energy expenditure (Nakagawa *et al.,* 2014; Nakagawa *et al.,* 2016; McCann *et al.,* 2021), suggesting integration of endocrine and metabolic pathways in skeletal height regulation.

LONGITUDINAL GROWTH TRAITS (BL AND TL)

Body length (BL) and tail length (TL) are economically important traits affecting carcass yield and breed characteristics. Tao *et al.* (2020) identified *LECT2* as a candidate gene for BL in neonatal sheep, while Li *et al.* (2020) reported *PDGF-D* as important for tail morphology.

In this study, a significant association for TL was detected on chromosome 12 near *OPTC*. Although *OPTC* was not enriched in GO or KEGG pathways, its positional overlap with the strongest SNP suggests potential relevance. For BL, no genome-wide significant genes were identified at peak regions, but suggestive loci included *IQGAP3, BCL6B, FGFR1, CDK6*,

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and *KLF4*. For TL, suggestive loci included *VAV3, DAB2IP, VAV2, FOXC1, EBF1, MITF, TCF4, PAK1, STK4, IQGAP1,* and *CREB3L4*.

Enriched functional categories included regulation of GTPase activity (*IQGAP1, IQGAP3, VAV2, VAV3, DAB2IP*), transcriptional regulation (*BCL6B, KLF4, FOXC1, EBF1, MITF, TCF4, CREB3L4)*, and protein phosphorylation (*FGFR1, CDK6, PAK1, STK4*). *IQGAP* scaffold proteins regulate cytoskeletal dynamics and cell proliferation (Hedman *et al.,* 2015), and *VAV* proteins regulate Rho/Rac-mediated signaling (Bustelo *et al.,* 2001). *FOXC1* controls endochondral ossification and chondrocyte maturation, influencing bone length (Almubarak *et al.,* 2024).

CREB3L4 was significantly enriched in GH synthesis and action, insulin secretion, cGMP–PKG signaling, cortisol synthesis, and aldosterone synthesis pathways. As a CREB/ATF family transcription factor interacting with androgen receptor signaling (Kim *et al.,* 2017; Ito *et al.,* 2024), *CREB3L4* may function as a central endocrine regulator influencing longitudinal skeletal growth.

BODY WIDTH TRAITS (HW, CHW, TW)

Body width traits influence carcass composition and productivity. Previous studies identified *ZNF395* associated with chest width (Erdenee *et al.,* 2020), and *TMEM50A*, *SCD5,* and *FKBP1A* associated with morphological traits (Kizilaslan *et al.,* 2022). Xu *et al.* (2021) reported *CERS6, BTG1, RYR3, SLC6A4, NNAT,* and *OGT* associated with tail shape traits.

In our study, significant loci were detected for HW (*KIDINS220, SCAMP2, ULK3, CPLX3, LMAN1L, CSK, CYP1A1*), ChW (*AHCYL1, STRIP1, ALX3*), and TW (*SH2D4B, CAPS2, ZPLD1*). GO enrichment revealed functional annotation for *CSK* (protein phosphorylation) and *ALX3* (transcriptional regulation). *CSK* is involved in diverse physiological processes including immune regulation and erythropoiesis (Zhu *et al.,* 2023), while *ALX3* regulates Wnt3a and VEGF signaling pathways essential for tissue differentiation (He *et al.,* 2019).

Moderately associated regions ($P \leq 0.001$) included genes involved in GTPase regulation (*FGD6, SYNGAP1, PLXND1, NF1, RAB3GAP2*), transcriptional control (*RUNX1, E2F8, ATF4, HOXA11, HOXA13, TBX6, UCP1, RELA*), and phosphorylation signaling (*EGFR, FYN, MYLK, PRKG1, PRKD1, MAPK3, PRKCB, PAK2, CAMK2G, BMPR1A, PDK1*). *KEGG* enrichment indicated roles for CAMK2G in Ca^{2+}-dependent endocrine pathways (Cao *et al.,* 2025), *UCP1* in apelin signaling and thermogenesis (Bonet *et al.,* 2017; Liu *et al.,* 2023), *PRKG1* in nutrient-related traits (Feng *et al.,* 2024), and *PRKD1* in tissue development (Waheed-Ullah *et al.,* 2024).

BODY GIRTH TRAITS (CHG, AG)

Li *et al.* (2013) reported *LRRC2* associated with chest girth. In our study, peak regions included *ROBO, SMC6, GEN1* (ChG) and *JUP, P3H4, FKBP10, NT5C3B, KLHL10, KLHL11, ACLY* (AG). *GEN1* participates in DNA repair during cell division (Benitez *et al.,* 2023), and *FKBP10* regulates collagen formation and skeletal integrity (Jarayseh *et al.,* 2025).

Suggestive loci included *ARID1B* (ChG) and *SMAD4, MAPK1, PDGFB, WNK1, MAPK9, PRKD2* (AG). *ARID1B* regulates gene expression via mTOR signaling (Lin *et al.,* 2023), *SMAD4* mediates TGF-β signaling in skeletal development (Pakravan *et al.,* 2022), and *MAPK1* and *MAPK9* participate in GH-related pathways (Zhang *et al.,* 2022). *PDGFB* supports

angiogenesis (Vazquez *et al.,* 2021), *WNK1* mediates macrophage lineage fidelity and tissue remodeling (Trzeciak *et al.,* 2025), and *PRKD2* is involved in Wnt-related skeletal signaling (Chen *et al.,* 2025). These findings suggest that girth traits may be influenced by coordinated regulation of proliferation, differentiation, and vascular development.

CANNON BONE CIRCUMFERENCE

Previous studies identified *FGF12* and *FTO* associated with cannon bone circumference (Dossybayev *et al.,* 2024), and GHR variants significantly associated with cannon circumference (Akhatayeva *et al.,* 2022).

In this study, peak regions included *ICE1, RPS5, RNF225, ZNF132, ZNF446, SLC27A5, TRIM28,* and *CHMP2A*. *RPS5* has been implicated in osteogenic differentiation (Guo *et al.,* 2026), and *SLC27A5* has been associated with growth traits in pigs (Liufu *et al.,* 2023).

Suggestive genes (*RASA2, PTK2, NR4A1*) were functionally relevant. *RASA2* regulates Ras signaling (Bell *et al.,* 2026), *PTK2* promotes osteogenic differentiation and bone formation (Wang *et al.,* 2025), and NR4A1 enhances WNT4/β-catenin signaling and bone regeneration (Gao *et al.,* 2024). KEGG analysis linked PTK2 to GH pathways and NR4A1 to aldosterone and oxytocin signaling, further supporting endocrine regulation of bone growth.

OVERALL INTERPRETATION

Collectively, our findings indicate that growth traits in Qira Black sheep are governed by a complex polygenic architecture involving MAPK/ERK signaling, Ras and Rho GTPase regulation, transcriptional control, hormone-related pathways (GH, GnRH, insulin, oxytocin, aldosterone), and metabolic regulation. Importantly, several biologically meaningful genes were identified within moderately significant regions ($P \leq 0.001$), underscoring the importance of integrating GWAS with functional annotation.

These results expand the understanding of the molecular mechanisms underlying morphometric traits in sheep and provide valuable candidate genes for future validation and marker-assisted selection programs.

5. CONCLUSION

In the present study, a genome-wide association analysis was conducted for 11 growth and conformation traits in 186 Qira Black sheep using the Illumina Ovine SNP50K BeadChip. Although no SNP reached the Bonferroni-corrected genome-wide significance threshold, multiple suggestive loci were identified across different chromosomes, supporting the polygenic nature of growth-related traits in this breed.

Candidate gene annotation and functional enrichment analyses highlighted genes involved in transcriptional regulation, protein phosphorylation, GTPase activity, and endocrine signaling pathways. In particular, genes such as *ROS1, VGLL2, CREB3L4, MAPK1, SMAD4, PTK2*, and *NR4A1* were associated with pathways related to growth hormone signaling, insulin secretion, and intracellular kinase cascades, suggesting potential roles in skeletal development and body conformation.

Overall, these findings provide preliminary insights into the genetic architecture underlying growth and developmental traits in Qira Black sheep. The identified candidate genes and enriched pathways offer valuable genomic resources for future validation studies

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and may contribute to marker-assisted selection strategies aimed at improving growth performance in this indigenous breed. However, further studies with larger populations and functional validation are required to confirm the biological roles of these loci.

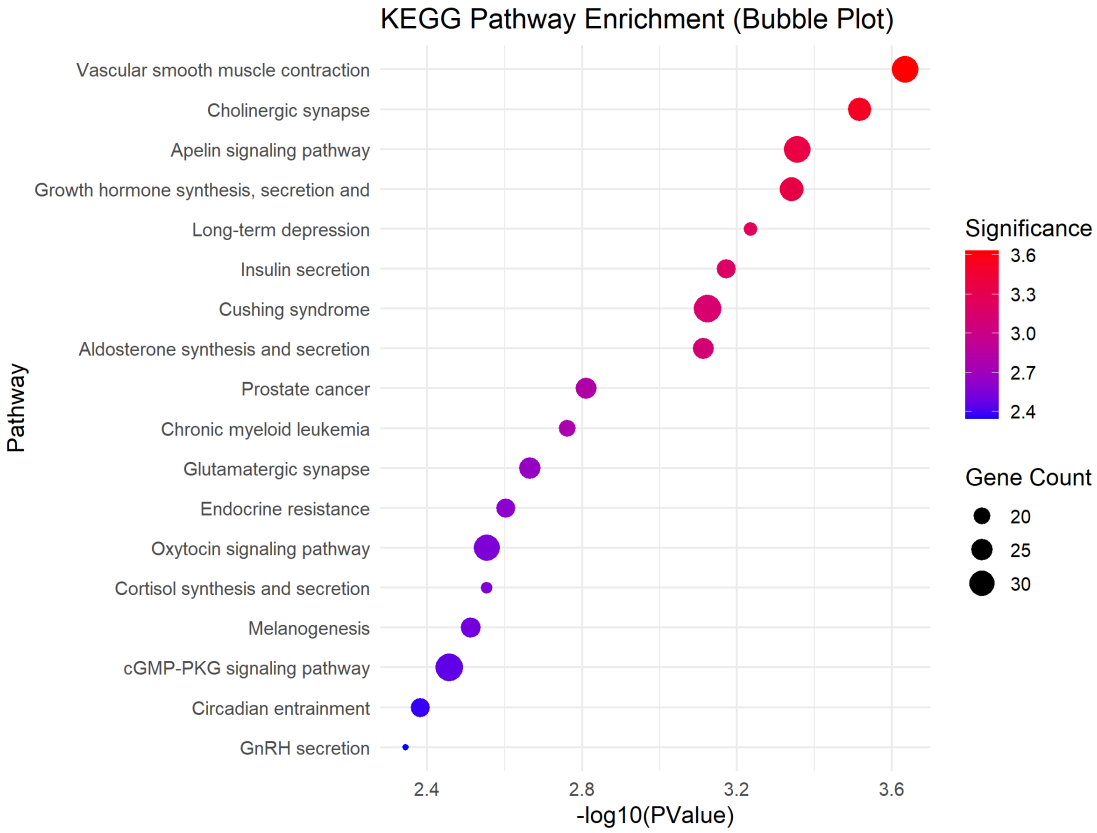
Authors contribution: Erkinbay Azbergenov conceived and designed the study, performed the main experiments, analyzed the data, and drafted the manuscript. Lingfeng Kong, Yuwei Peng, Yanhao Li, and Lijun Zhu participated in experiments, data collection, and analysis. Ruizhi Yang, Fuming Kou, Yaxuan Liao assisted with methodology, validation, visualization, and resources. Tao Jiang and Shudong Liu supervised the project, provided funding and resources, revised the manuscript critically. Tao Jiang and Shudong Liu are the co-corresponding authors. All authors read and approved the final manuscript.

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Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request. The data are not publicly available due to ownership and commercial considerations.

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59. *Figure 5.* KEGG pathway enrichment analysis of candidate genes associated with growth and development traits in Xinjiang Qira Black Sheep. The top significantly enriched pathways are presented. Bubble size represents the number of genes involved in each pathway, and color intensity indicates the level of significance expressed as −log10(P-value).
60. *Table 3.* Gene Ontology (GO) biological process enrichment analysis of GWAS-associated genes
61. **Candidate overlap (k/104)**

62. regulation of GTPase activity
63. protein phosphorylation
64. regulation of transcription by RNA polymerase II
65. activation of GTPase activity
66. *Table 3. The enrichment analysis was performed using 2,551 genes identified from the 11 sheep traits. "Gene Count" indicates the number of genes from this set that mapped to the specific GO term. "Candidate overlap (k/104)" represents the number of prioritized candidate genes (n = 104) found within each enriched GO term. "Cluster Enrichment" represents the fold enrichment score. FDR, false discovery rate.*
67. **Table 4.** KEGG pathway enrichment analysis of GWAS-associated genes.
68. **Candidate overlap (k/104)**
69. **Growth hormone synthesis, secretion and action**
70. **Insulin secretion**
71. **Apelin signaling pathway**
72. **Vascular smooth muscle contraction**
73. **cGMP-PKG signaling pathway**
74. **GnRH secretion**
75. **Cortisol synthesis and secretion**
76. **Aldosterone synthesis and secretion**
77. **Oxytocin signaling pathway**
78. *Table 4. Enrichment analysis was performed using the 2,551 GWAS-associated genes. "Gene count" indicates the number of genes from this set mapped to each KEGG pathway. "Candidate overlap (k/104)" indicates the number of prioritized candidate genes (n=104) included in each enriched pathway. P-values were corrected for multiple testing using FDR.*