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Screening of molecular markers associated with hornless traits in Qira black sheep

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Abstract

Hornless trait in sheep is one of the important traits affecting their economic production efficiency. In order to screen the molecular markers related to hornless trait in Qira black sheep, we used Illumina Ovine 50 K SNP chip data of 189 Qira black sheep, which were divided into two groups based on their horned and hornless traits. GWAS analysis of individuals of interest. Used the horned trait group as the reference group, and performed xp-EHH analysis with the hornless group. The hornless group was subjected to iHS and Pi analyses. After annotating the relevant candidate regions based on the sheep 4.0 genome microarray data. 681 candidate genes were obtained from the xp-EHH analysis, 87 candidate genes from the iHS analysis, and 2,171 candidate genes from the Pi analysis. A total of 19 candidate genes were obtained from further analysis of the candidate regions. Candidate genes were analyzed by GO and KEGG, and *RXFP2*, *TNS3*, *EYA2* and *OCSTAMP* genes were found to be associated with horned traits in Qira black sheep. Further PCR validation of *RXFP2* revealed that loci 10: 29,501,280 ($P=0.002$), 10: 29,501,431 ($P=0.003$) and 10: 29,051,333 ($P=0.0001$) were significantly associated with hornless trait in Qira black sheep.

Highlights

- In the present study, through the analysis of selection signals among the horned and hornless populations of Qira black sheep, we screened out the candidate molecular markers related to the hornless trait of Qira black sheep and found the relevant mutation sites, which verified the important role of the *RXFP2* gene on the hornless trait, and also screened out the other candidate genes related to the hornless trait. The results provide a basis for the selection and breeding of the hornless group in Qira black sheep.

Keywords Qira black sheep, Hornless trait, Candidate gene, Selection signal analysis

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Introduction

Sheep horn phenotypes are categorized according to the presence or absence and number of horns into hornless, two-horned and multi-horned phenotypes [1]. When humans domesticated sheep, they simultaneously selected for traits such as coat color, wool type, and horn type. The horn was one of the first traits humans began to study. Having horns is considered an important trait for mammals, but hornless herbivores are more in line with the current requirements of intensive livestock management [2]. In modern intensive production, fights between horned individuals often result in injury or even death of the individuals [3], increasing veterinary costs, and the number of horned sheep housed in the same barn is less than the number of hornless sheep, which increases farming costs. Various physical, chemical and surgical [4] methods are often utilized to remove horn buds from young individuals [5]. However, these methods are not only wasteful of labor and material resources, but also cause short-term or long-term pain [6] to the young animals, causing unnecessary stress and thus affecting the growth and development of the animals [7]. Therefore, in order to better intensive management and improve economic efficiency, selection of hornless populations by identifying genetic loci controlling horned/hornless traits and screening reliable molecular markers for hornless traits is an effective way to obtain hornless individuals [1].

In most modern breeds, selection of hornless sheep is of great interest, not only from breeding aspects to select hornless breeds, but also from some local breeds [3]. Hornless sheep began to appear in domesticated sheep from the 16th century [1], and it has been reported that the hornless trait is recessive [8], while the genes associated with horn growth are additive, Ho+/Ho + = horned; Ho+ / HoP = scurred; HoP / HoP = hornless [9]. However, the role of genes varies from species to species and Pickering et al. showed that there is no homology between the position of OAR10 for sheep horn/hornless and the position of the *BTA1* for bovine horn/hornless, suggesting that the same traits in the two species are determined by different genes [10]. The position of the horn/hornless locus in Soay sheep was determined to be on chromosome OAR10 in the approximately 29.4 Mb of the 250 kb region [10]. Dominik [11] et al. mapped the hornless trait to a SNP at Mb position 29.38 on sheep chromosome OAR10 using Illumina Ovine 50 K SNP chip data in Australian Merino sheep, the nearest gene being *RXFP2*. Kijas [12] et al. used Illumina Ovine 50 K SNP chip data to genetically analyze 2,819 sheep from 74 different breeds and found that the strongest selection signal associated with the hornless trait was identified immediately adjacent to *RXFP2* at position 29.54 Mb on chromosome OAR10. The above findings suggest that *RXFP2* is a breeding target for selecting hornless breeds.

It was found that most of the Qira black sheep rams had horns, and most of the ewes had hornless or had undeveloped small horns. In this study, 189 Qira black sheep were selected and divided into horned ($n = 41$) and hornless ($n = 148$) groups according to traits, and molecular markers related to the hornless trait in Qira black sheep were screened out by selective signal analysis based on their Illumina Ovine 50 K SNP chip data.

Materials and methods

Materials

We collected venous blood from 189 healthy Qira black sheep ewes aged between 1 and 3 years old under uniform housed management, including 41 horned and 148 hornless sheep(Fig. 1), from Xinjiang Hetian Qira Jinken Agricultural and Animal Husbandry Science and Technology Co. Genotyping was performed based on Illumina's SNPline genotyping platform from Beijing Compassion Biotechnology Co. Ltd. The dataset

Fig. 1 Horned and hornless qira black sheep photos

consisting of data from 189 individuals with individual SNPs was obtained. Quality control was performed using PLINK v1.90 [13], with maximum missing rate (max-missing) < 10%, SNP loci with > 90% detection rate, and minimum allele frequency (MAF) > 0.01 (P-value of Hardy-Weinberg equilibrium test is > 10^-6 -hwe); using the --min-alleles 2 ---max-alleles 2 to remove multiple alleles, and the --min-alleles 2 ---max-alleles 2 to remove multiple alleles, and the --min-alleles 2 --max-alleles 2 to remove multiple alleles. alleles 2 to remove multiple alleles and allelic deletions. Ultimately, 53,093 SNPs were available for subsequent analysis.

Methods

GWAS

Correlation analysis of horn type traits of Qira black sheep was performed using TASSEL 5.0 software based on generalized linear mixed model (GLM). The model's mathematical academic expression is given as:

$$\log\left(\frac{P(y_i=1)}{1-P(y_i=1)}\right)=\beta_0+\beta_g\cdot \mathrm{SNP}_i+\beta_c\cdot \mathrm{Covariates}_i+u_i$$

yi∈{0,1}: phenotype of individual i (0 = hornless, 1 = horns).

βg: fixed effect of the target SNP (The contribution of the locus to the phenotype).

ui ~ N (0,σ2K): random effects, K is the kinship matrix capturing genetic correlations between individuals.

Thresholds were determined by the Bonferroni correction method, and SNPs were considered to be strictly genome-wide significant at $P < 0.05/N$ and potentially significant at $P < 0.1/N$ [14]. In this study, the thresholds of significance and potential significance were 6.0261 (-log10 (0.05/53093)) and 5.7250 (-log10 (0.1/53093)), respectively.

iHS analysis

IHS analysis is an analysis of the probability that a haplotype remains identical at a given locus, the rate of decay of a haplotype at a locus. If a haplotype has a high iHS value at the locus, it means that the haplotype is tightly chained to SNPs at the locus. The iHS method is suitable for detecting natural and artificial selection signals and can be used to compare genetic variation and differences in selection pressure between haplotypes in the same population [15]. The Qira black sheep hornless group was individually analyzed by iHS, using a single marker locus to replace the core haplotypes in the EHH statistics, defining them as core loci, calculated as:

$$iHS=\frac{uniHS-mean\left(uniHS\,|p_s\right)}{sd\left(uniHS\,|p_s\right)}$$

The uniHS is set:

$$uniHs=In\left(\frac{iHH_A}{iHH_D}\right)$$

IHH refers to the integration of genetic distances of EHH (integrated EHH), a represents the ancestral (progenitor) allele, and D represents the new mutant (derived) allele.

XP-EHH analysis

The xp-EHH is used to compare selection signals between populations. Suitable for comparing differences in genetic variation and selection pressure between two populations. The xp-EHH analysis, which is based on LD analysis but with a wider region of selection [16], is a method of comparing extended haplotype purity between populations to detect points of selection that are segregating in a population [17]. Compared with the iHS method, the xp-EHH method has high sensitivity and accuracy and can detect selection signals at the population level while having less impact on gene flow and mutation rates. The positive xp-EHH indicates that the haplotype is affected by selection in a horned population, and a negative xp-EHH value indicates that the haplotype is affected by selection in a hornless population. We did xp-EHH analysis of Illumina Ovine 50 K SNP chip data from horned and hornless populations of Qira sheep [18], and the xp-EHH statistic was calculated as:

$$xpEHH=\frac{unxpEHH-mean\left(unxpEHH\right)}{sd\left(unxpEHH\right)}$$

The unxp EHH is set:

$$unxpEHH_{\mathrm{scores}}=In\left(\frac{iES_{pop1}}{iES_{pop2}}\right)$$

iESpop1 integrates EHH genetic distance statistics, and iESpop2 integrates genetic distances from EHH statistics of horned populations.

Pi analysis +

When a region on a gene sequence is subjected to selection, the region, as well as its chain of regions, shows a decrease in polymorphism along with an increase in purity due to selective scavenging. Therefore the region of the genome under selection can be inferred by detecting the nucleotide diversity of the genome [19]. The nucleotide diversity is a measure of the level of variation within a genome, also known as nucleotide diversity. It indicates the average number of different alleles per locus in a given DNA region or genome. A higher value of Pi indicates a higher level of genetic diversity in that genome. Hornless population chip data of Qira black

sheep were analyzed for population nucleic acid diversity using VCFTOOLS. We set the sliding window of Pi analysis to 50 kb and the step size to 25 kb, and the SNP loci in the top 5% of the Pi values were used as significant candidates for analyzing polymorphisms in the hornless population of Qira black sheep.

$$\pi = \frac{\sum_{i<j} d_{ij}}{\binom{n}{2} \cdot L}$$

n: number of samples; d_{ij}: number of sites of difference between the ith and jth sequence; L: total length of the sequence (or number of valid sites analyzed); $\binom{n}{2}$: number of two-by-two combinations of samples (n(n-1)/2)

Candidate gene screening

We positively selected the top 5% SNP loci for XP-EHH, IHS and pi analysis values as selection sites to identify regions under selection in the genome, and for each of the scans the analysis results were visualized and analyzed using -R. All obtained SNPs were based on the Sheep Oar_v4.0 (https://www.sheephapmap.org/) for the Annotation. Genes were functionally analyzed with reference to the NCBI database (http://www.ncbi.nlm.nih.gov/gene), the Gene Ontology database (GO, http://geneontology.org) using the DAVID tool (http://david.Abcc.ncifcrf.gov/) [20] and Kyoto Encyclopedia of Genes and Genomes (KEGG, Kyoto Encyclopedia of Genes and Genomes) [21] for analysis. Visual intersection analysis was performed using Draw Venn Diagram (Draw Venn Diagram (ugent.be)) for individual analysis annotations.

Genomic DNA extraction

DNA was extracted from the collected blood using FinePure Blood/Cell/Tissue Genomic DNA Extraction Kit (Centrifugal Column Type) (Jifan Biotechnology Co., Ltd., Beijing, China) according to the kit manufacturer's instructions, and the DNA quality was tested by agarose gel electrophoresis using 1.5% agarose gel dissolved in 1X TBE buffer and visualised using a fully automated gel imager (Hangzhou HaiPu Instrument Co. Ltd, Hangzhou, China) for visualisation. The concentration of the extracted DNA was measured using a nucleic acid protein detector (Shenzhen Enke Biotechnology Co., Ltd., Shenzhen, China).

Table 1 Candidate gene PCR region primer related information

Region	Primers	Product Length (bp)	Annealing Temperature
RXFP2	F: TGACTCCAAACCAGAAGATTCC R: TGCTGCAGAGATAGAGATCCAC	962	64.5℃

Polymerase chain reaction (PCR)

The *RXFP2* (containing part of the intron) gene was amplified using PCR (Life Pro Thermal Cycler, Hangzhou, China) and the optimal annealing temperature was determined using gradient thermal gradient PCR. A PCR reaction system of 2ul of 30～100ng/ul DNA, 2ul each of 10 μm forward and reverse primers, and 25ul of 2×Taq PCRmix (with dye/blue) was used for a total of 50ul. Amplification was carried out using the following procedure: pre-denaturation at 98 °C for 3 min, performing the first set of cycles under the conditions of denaturation at 98 °C for 30 S, annealing at 52 °C for 30 S, and extension at 72 °C for 30 S. The second set of cycles was performed under the conditions of denaturation at 98 °C for 30 S, annealing at 64.5 °C for 30 S, and extension at 72 °C for 30 S. The final extension of the reaction was carried out at 72 °C for 5 min (Table 1) [22]. PCR product quality was checked using 1X TAE 1.5% agarose gel electrophoresis.

Sequencing procedure

After PCR product preparation and purification, PCR products were subjected to one-way passaging using the Sanger sequencing method at Sango Bioengineering (Shanghai) Co. PCR product sequences were tested for mutant sites using the NCBI online website (https://blast.ncbi.nlm.nih.gov/Blast). Allele frequencies and genotype frequencies were calculated by direct counting method, and the correlation between the *RXFP2* and the hornless trait was calculated using SPSS based on the statistical analysis.

Results

Quality control

After initial quality control of the Illumina Ovine 50 K SNP chip data from 189 Qira black sheep according to the standards, 186 sheep met the quality control criteria. 53,093 SNPs from 186 Qira black sheep were used for subsequent analysis after quality control. Among them, there were 40 horned sheep (W) and 146 hornless sheep (A).

Results of GWAS

A total of 53,093 SNPs were revealed by GLM analysis using the common GWAS model, and one significantly associated SNP locus, located on chromosome 27, which was not reported to be associated with sheep horn traits (OARX_22360871.1), was found in the Qira black sheep horn trait using a threshold of 6.0261 (-log10 (0.05/53093)) (Fig. 2).

Results of iHS analysis

The resultant values with iHS > 0.5 are regarded as candidate regions, which were annotated to obtain 87

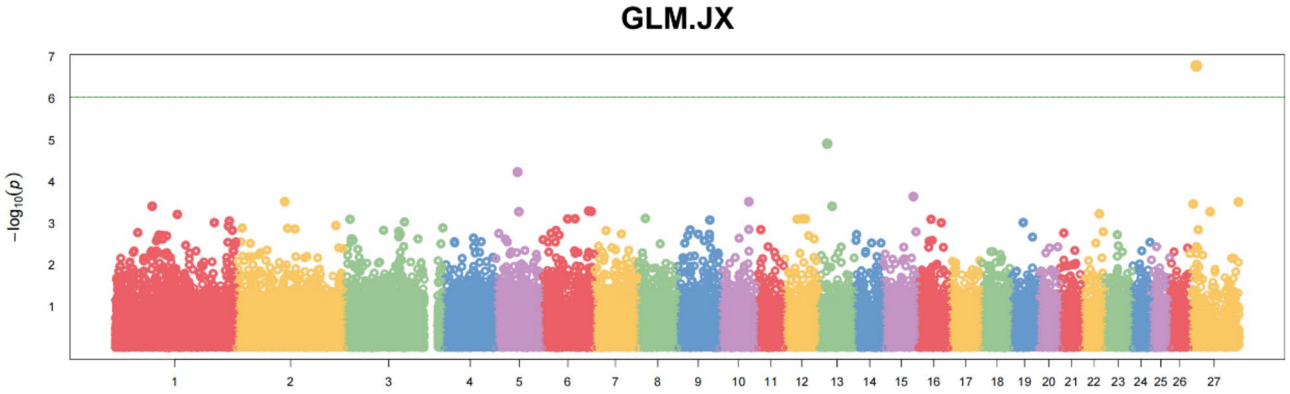

Fig. 2 Manhattan plot of genome-wide association analysis associated with angular phenotype. The green line is the significant association threshold. The vertical axis is the -log10(p) value, horizontal axis is chromosome number

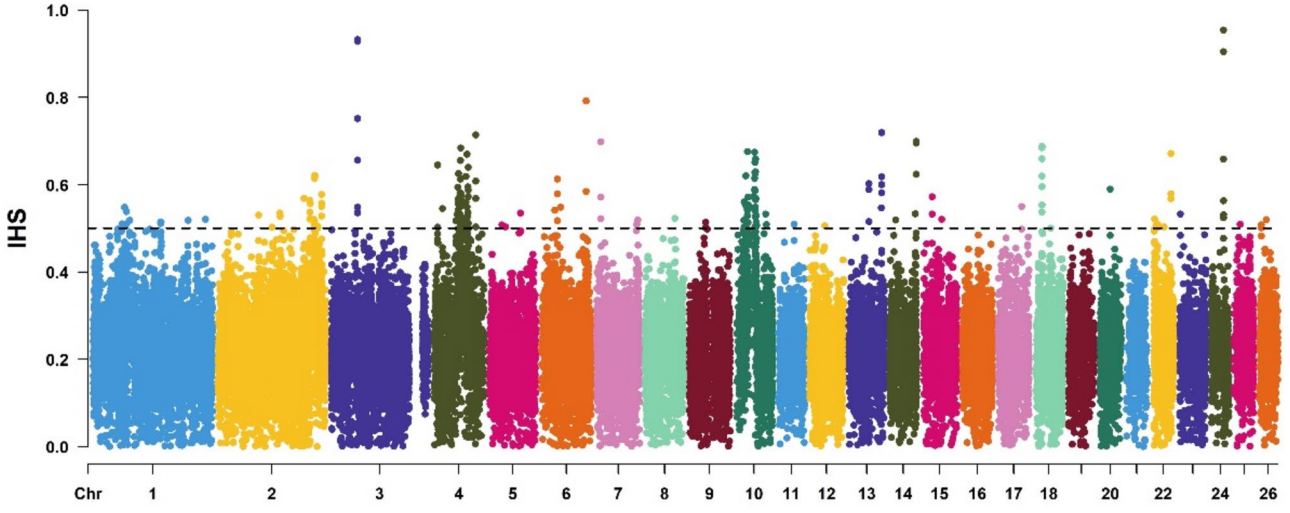

Fig. 3 Manhattan plot of the results of iHS analysis of the hornless population. Note: Different colors in the figure represent different chromosomes, and the value of the black dotted line in the figure is 0.5

candidate genes, which were mainly distributed on chromosomes 2, 4, and 10 (S1 Table; Fig. 3).

Results of xp-EHH analysis

By analyzing the xp-EHH of 40 horned and 146 hornless Qira black sheep, we annotated and analyzed the top 5% of both ends of the results as the region of selection, and identified 681 differential genes (S2 Table; Fig. 4).

Results of Pi analysis

Nucleotide diversity was analyzed in the antlerless population of Qira black sheep, and the top 5% of the results were used as candidate regions, which were annotated to yield 2,171 candidate genes (S3 Table; Fig. 5).

Results of candidate gene screening

The xp-EHH analysis of Illumina Ovine SNP 50 K chip data lines from the hornless population of Qira black sheep, and intersection analysis of iHS and Pi analyses of the hornless population revealed that there were 19 candidate genes associated with the hornless trait (Fig. 6; Table 2).

Candidate gene enrichment analysis

In this study, we analyzed the biological functions of the candidate genes and identified their enrichment pathways (Fig. 7). *OCSTAMP* is involved in the osteoclast differentiation pathway, and *TNS3* and *OCSTAMP* are jointly involved in the regulation of the extracellular matrix organization pathway. *DRD2* is involved in the regulation of neurotransmitter transport pathway. *PIBF1* and *RXFP2* are involved in the regulation of the progesterone-mediated oocyte maturation pathway. *RXFP2* and *EYA2* are involved in the regulation of male gonadal development pathway. *VKORC1L1* is involved in the regulation of oxidoreductase activity pathway. *PIBF1* and *RXFP2* are involved in the progesterone-mediated oocyte maturation pathway. *RXFP2* and *EYA2* are involved in

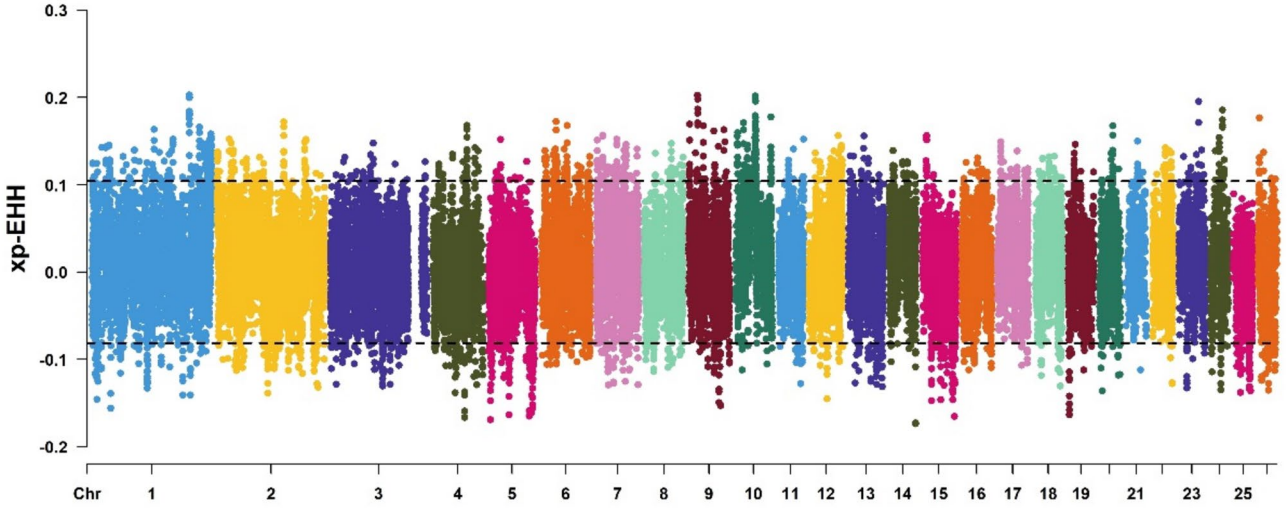

Fig. 4 Manhattan plot of XP-EHH analysis results. Note: Different colors in the figure represent different chromosomes, and the black dotted line in the figure represents the value of 5% of the absolute value of xp-EHH

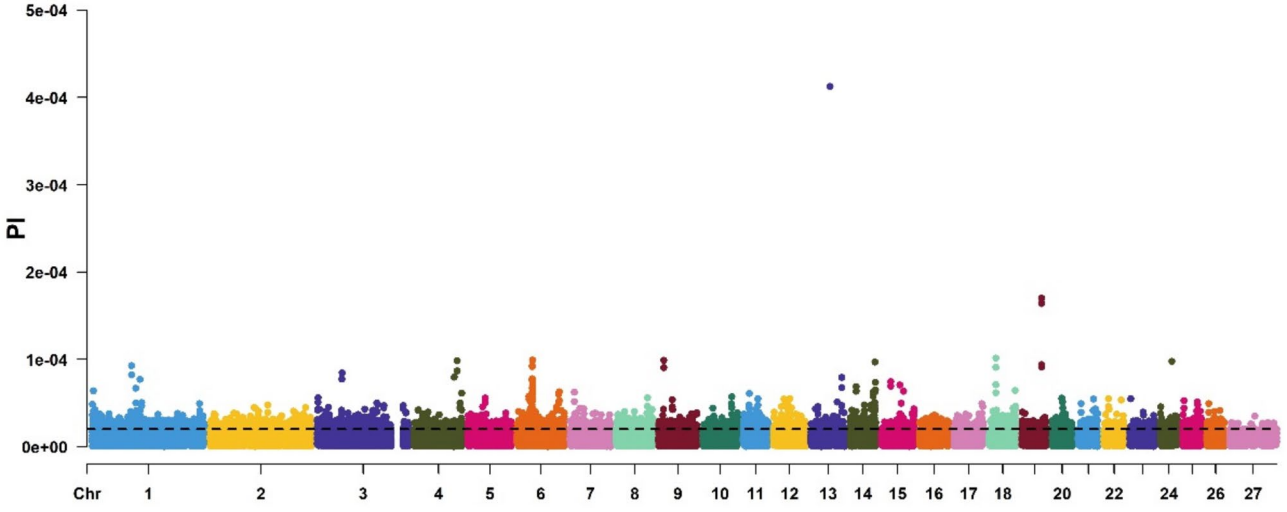

Fig. 5 Manhattan plot of the Pi analysis results of the hornless population. Note: Different colors in the graph represent different chromosomes, and the black dotted line in the graph represents the top 5% of values with Pi = 0.0000246

the male gonadal development pathway. *VKORC1L1* is involved in the oxidoreductase activity pathway.

DNA extraction as well as PCR results

As shown in Fig. 8, it can be clearly seen that the DNA bands are clear and free of contamination, and after the detection of nucleic acid detector, it was found that the OD260 nm/OD280 nm was at 1.9 ~ 2.0, and the integrity of the DNA was in accordance with the experimental requirements. As shown in Fig. 9, the PCR product bands were bright and clear and consistent with the expected size, which met the sequencing requirements.

Association analysis of the RXFP2 with the hornless trait

As shown in Fig. 10, the 3 mutations were observed in the 962 bp product generated by PCR amplification of the *RXFP2*. The chi-square test values for the association analysis of these mutant loci with hornless traits in Qira black sheep are shown in Table 3. The 1 mutation site was 10:29501431, where the resulting T to C mutation changed serine to proline ($P = 0.002$). The 2 mutation site is 10:29501333, where the T to C mutation produced is a synonymous mutation in leucine ($P = 0.003$). The 3 mutation site is 10:29501280, where the T/C mutation is synonymous with alanine ($P = 0.0001$).

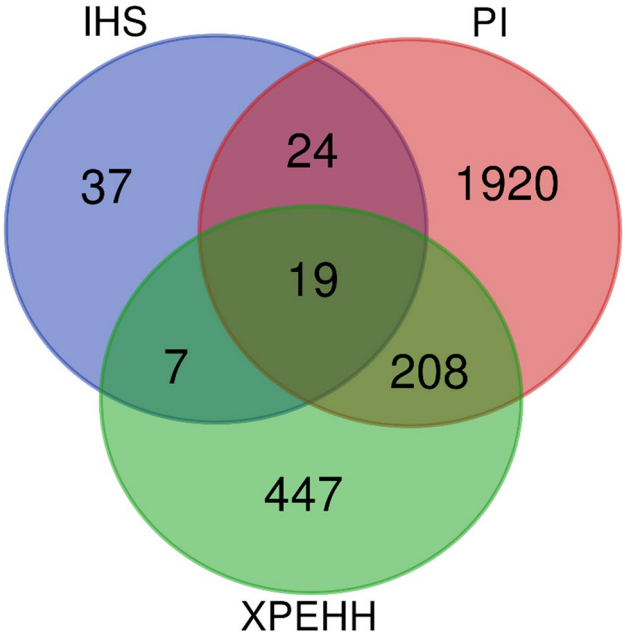

Fig. 6 Candidate gene intersection map. Note: The green area in the figure shows the candidate genes obtained from xp-EEH analysis, the pink area represents the candidate genes obtained from Pi analysis, and the blue area represents the candidate genes obtained from iHS analysis. The number of candidate genes shared by the three analyses was 19, with 26 (7 + 19) intersecting genes from iHS and xp-EHH analyses, 227 (208 + 19) candidate genes shared by Pi and xp-EHH analyses, and 43 (19 + 24) candidate genes shared by Pi and iHS analyses

Discussion

Hornless groups of sheep are more in line with the management needs of modern animal husbandry, which can reduce the livestock production and management costs, the survey showed that the ewe part of Qira black sheep is horned trait, the development of the horn involves hundreds of genes, and its precursor cell patterning and differentiation occurs in the early stage of embryogenesis [23], the sheep horn belongs to the hole horn, also known as the virtual horn, from the inside to the outside from the dermis formed by the bone heart and the epidermis formed by the keratin sheath composition. The bone core is hollow with a large number of capillaries and nerve fibers, the keratin sheath is hard and protects the bone core. Horn growth begins with the production of dermally derived horn buds at the upper end of the frontal bone, formed by differentiation of mesodermal tissues of the epidermal cuticle, which eventually combine with the skull to form horn genesis [24]. The growth of the bony core depends mainly on the continuous thickening of the osteogenic tissue bone at the tip of the core and the outer layer of the core. This specialized horn structure lays the foundation for the curvature of the horn during growth and the diversity of horn shapes. We hypothesized that genes related to osteoblast growth, osteogenic differentiation, and bone production are closely associated with the growth and development of sheep horns.

In this study, 186 Qira black sheep were analyzed by GWAS and selection signals. The GWAS results failed to directly identify significant candidate genes associated with the horn trait in Qira black sheep. It indicated that the hornless trait of Qira Black sheep was subjected to strong artificial selection during domestication, and the primary effector genes were fixed in the breed. The fixation of the main effector genes for the hornless trait was a direct result of artificial selection, which led to the failure of GWAS, but the analysis of the selection signals could clearly reveal the history and biological importance of

Table 2 Candidate gene values analyzed for each

Chromosome	Start site	End site	IHSvalue	PIvalue	XP-EHHvalue	Gene
12	35,212,512	35,212,970	0.50543	0.0000421	0.106007	*CCDC181*
13	75,376,077	75,376,224	0.719212	0.0000675	0.0958591	*SNORA79*
4	64,836,318	64,836,375	0.575656	0.0000269	0.102136	*CCDC129*
24	27,869,198	27,869,219	0.953963	0.0000977	0.185459	*VKORC1L1*
15	23,399,833	23,400,099	0.572578	0.0000743	0.147295	*ANKK1*
24	27,879,763	27,879,926	0.953963	0.0000977	0.185459	*GUSB*
12	35,179,687	35,180,505	0.50543	0.0000519	0.106007	*BLZF1*
13	46,225,254	46,226,024	0.602356	0.000412536	0.103755	*PRP*
13	75,328,375	75,328,455	0.719212	0.0000791	0.0958591	*EYA2*
14	61,313,409	61,313,954	0.699185	0.0000736	0.173873	*USP29*
10	48,060,989	48,061,087	0.512801	0.0000331	0.195685	*PIBF1*
15	23,419,863	23,420,781	0.572578	0.0000743	0.147295	*DRD2*
23	2,551,266	2,551,577	0.53273	0.0000548	0.0996077	*ZNF516*
10	29,456,118	29,457,146	0.571361	0.000025	0.120272	*EEF1A1*
2	223,588,798	223,588,843	0.621112	0.0000287	0.11382	*SGPP2*
12	35,179,891	35,179,893	0.50543	0.0000519	0.106007	*NME7*
13	74,793,765	74,794,094	0.546901	0.0000468	0.0994722	*OCSTAMP*
10	29,458,217	29,458,219	0.571361	0.000025	0.120272	*RXFP2*

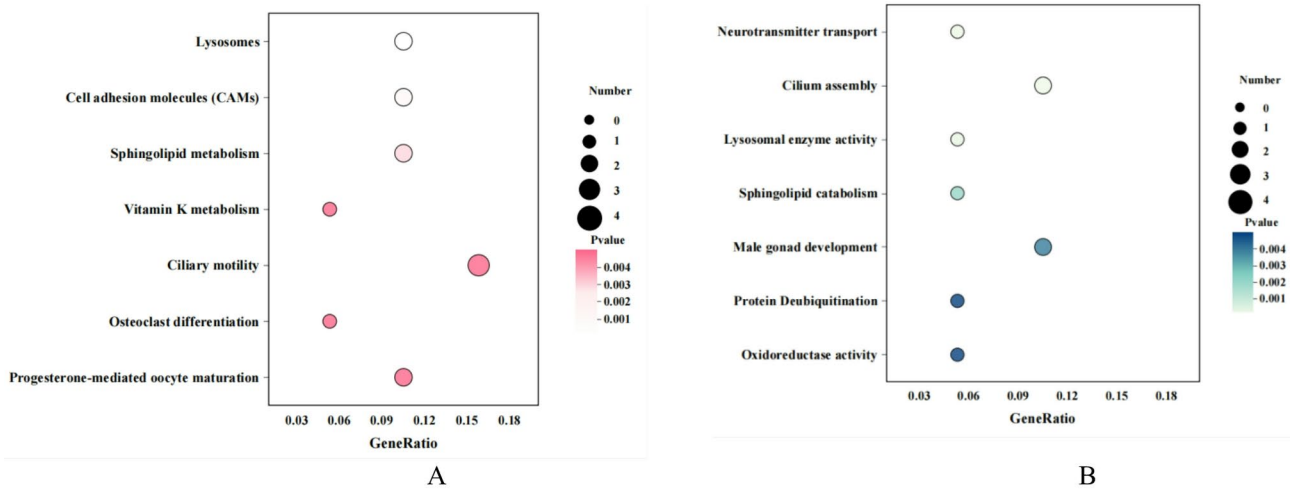

Fig. 7 Enrichment analysis bubble plots. **A** is the result of KEGG enrichment analysis, **B** is the result of GO enrichment analysis

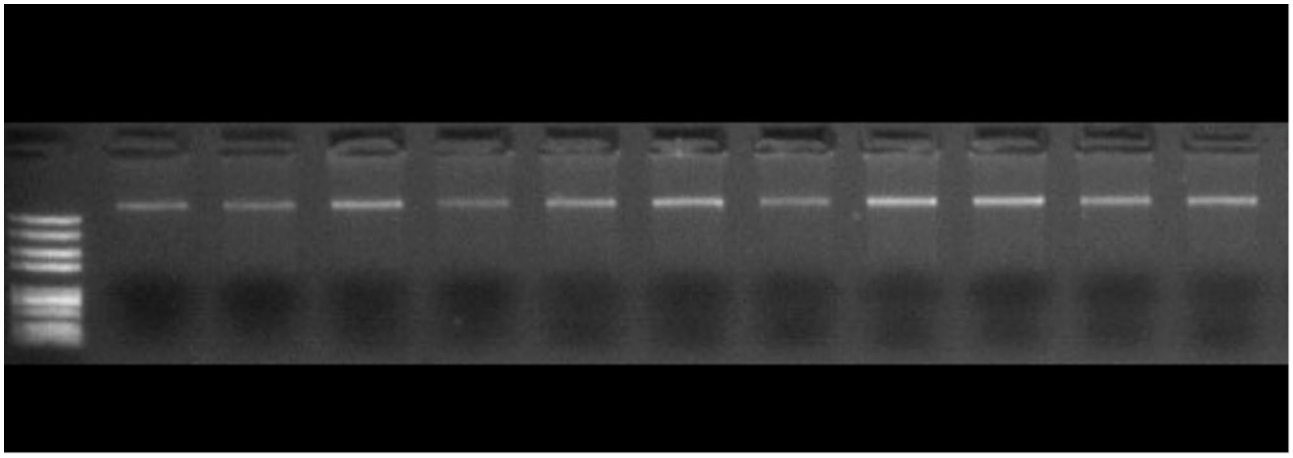

Fig. 8 DNA extraction results

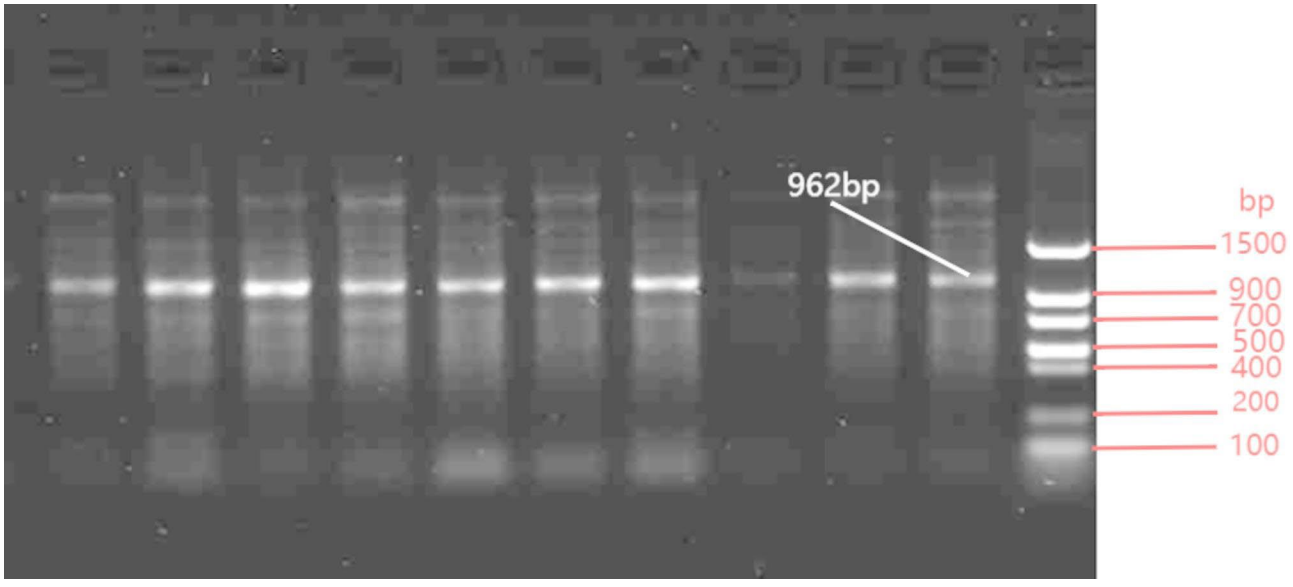

Fig. 9 PCR amplification results

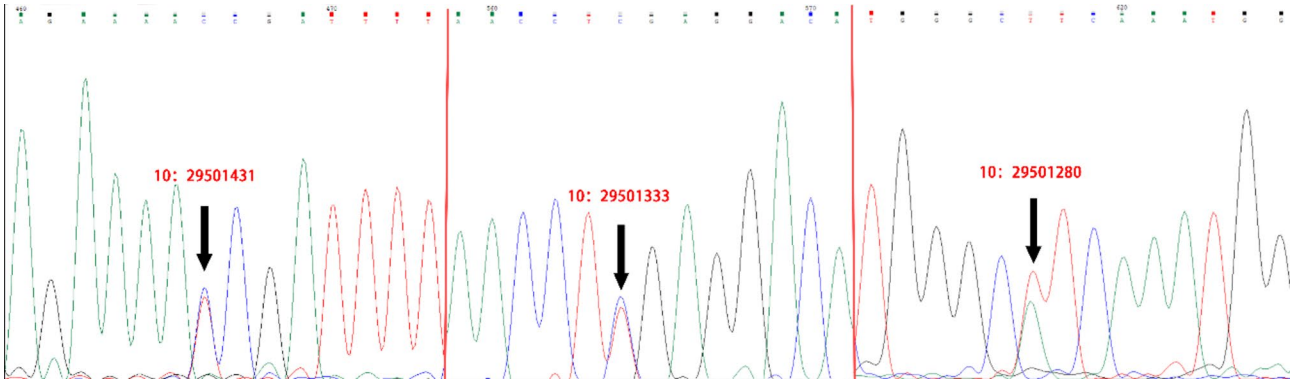

Fig. 10 Mutation site detection

Table 3 Relationship between candidate gene genotypes and related phenotypes

Gene	Mutant site	Genotypes	Phenotypic trait	Percentage of phenotypes (%)	Chi-Square test (*p*-value)
RXFP2	10: 29,501,431 T> C	TC	Y	80	0.002
			W	20	
	10: 29,501,333 T> C	TC	Y	65	0.003
			W	35	
	10: 29,501,280 T> C	TC	Y	7	0.0001
			W	93	

Note: Y stands for horned individuals, W stands for hornless individuals

the selection. In further XP-EHH analysis of the 53,093 SNPs in the horned and hornless populations of Qira black sheep after quality control, we found the *RXFP2* associated with the horn trait in the candidate regions at both ends, and in order to further determine the selected characteristics of the gene, we performed IHS as well as PI analysis of the hornless population of Qira black sheep, and the results of the assay showed that the *RXFP2* showed strong selection signal in the selection signal The strong selection signal of hornless breeds (small-tailed cold sheep) in the region of the *RXFP2* and its absence in grassland Tibetan sheep in the study of Pan [25] et al. indicated that this locus was the main target of artificial selection during domestication. Then, the selection signal results are plausible and reflect the targeted selection on the *RXFP2* by historical artificial selection.

The *RXFP2* (Relaxin family peptide receptor 2) is located at position NC_056063.1 (29499436.29561401) on chromosome 10 in sheep. Getachew [26] et al. showed that the *RXFP2* could be a candidate gene for the hornless trait in Tibetan sheep, Qinghai wool-meat-combining fine-wool sheep and alpine Merino sheep. Dario et al. localized the hornless phenotype of Soay sheep to chromosome 10 [27]. Kijas [28] et al. identified the hornless phenotype of Soay sheep on the chromosome 10 by a high-density NAVAJO-Churro sheep chip for genome-wide association analysis localized the hornless locus between 29.3 and 29.5 Mb on chromosome 10 in sheep. In this study, the differential gene *RXFP2* was annotated on chromosome 10 between 29,458,217 ~ 29,562,572. One high-frequency mutation site (10:29501310), and two low-frequency mutation sites (10:29501431 and 10:29051333) revealed by amplification sequencing of the *RXFP2*. This is consistent with the findings of Kijas et al. Suggests that the *RXFP2* is an important candidate for influencing the production of hornless traits in Qira black sheep.

We also identified *TNS3*, *EYA2* and *OCSTAMP* genes associated with bone growth, which also show strong selection in hornless populations and are thought to be associated with the formation of hornless traits in Qira black sheep.

The *TNS3* (Tensin 3) is located at position NC_056057.1 (76390518.76613933) on chromosome 4 in sheep, and the expression level of *TNS3* determines the shape of the cell and the reorganization of the cytoskeleton during osteogenic differentiation. *TNS3* truncation experiments showed that the *TNS3* was essential for optimal osteogenesis, and all structural domains were shown to be essential. Pull-down and immunocytochemistry experiments showed that *TNS3* mediates osteogenic differentiation through RhoA [29]. The *TNS3* can regulate osteogenesis, and its expression has an effect on the growth and development of sheep horned heart, which is inseparable from the regulation of bone growth.

The *EYA2* (EYA transcriptional coactivator and phosphatase 2) is located at position NC_056066.1 (75493774.75753318) on chromosome 13 in sheep, which was associated with body growth traits in Locally Adapted Egyptian Barki Sheep. In Ethiopian sheep, *EYA2*

is considered a candidate for tendon, bone and cartilage embryonic development [30] also a candidate gene for weaning weight. As they are associated with biological processes such as metabolism, body growth, organ morphogenesis, skeletal muscle development as well as cell proliferation and differentiation [31]. *EYA2* has been shown to be closely associated with the biological processes of rhabdomyosarcoma tissue development, muscle cell and skeletal muscle cell differentiation [30], and its regulation of skeletal muscle cell differentiation regulates skeletal growth and development, while growth regulation of the horns has been closely linked to skeletal growth hypothesize that the horn is closely related to skeletal growth and development. We hypothesized that the skeletal growth as well as the development of the Qira black sheep is correlated with the angular trait.

The *Ocstamp*, NC_056066.1 (75223179.75230538) located on chromosome 13 in sheep, is an osteoclastogenic gene for the gene osteoclast-stimulating transmembrane proteins [32], which are associated with osteoclast differentiation [33]. The rapid maturation of the bone is attributed to the efficient bone mineralization and reconstruction process realization, said bone reconstruction process involves the deposition of new bone and resorption of old bone [34]. Cell-cell fusion is another unique maturation process of osteoblasts accompanied by multinucleation, which is reportedly controlled by seven transmembrane proteins expressed by dendritic cells (Dcstamp) and osteoblast-stimulated transmembrane protein (*Ocstamp*) [35]. Key regulatory role as a downstream regulator of RANKL signaling, *OC-STAMP* plays a key regulatory role during the osteoclast fusion phase [36]. The horn growth of sheep can't be separated from the regulation of osteoclasts.

In the absence of significant results from GWAS analysis, this study analyzed the haplotype and nucleotide diversity in order to trace the candidate genes associated with the hornless trait in Qira black sheep. From the point of view of horn bone development, the present study concluded that there is a correlation between the *RXFP2*, *TNS3*, *EYA2* and *OCSTAMP* and the growth of horns of the black sheep in Qira black sheep and verified the correlation between the *RXFP2* and the hornless trait.

Conclusion

GWAS analysis and selection signal analysis of horn traits in Qira black sheep screened *RXFP2*,*TNS3*, *EYA2* and *OCSTAMP* associated with hornless traits. The PCR validation of the main effector gene, *RXFP2*, identified three mutation loci significantly associated with hornless traits in Qira black sheep. This provides a theoretical basis for the selection and breeding of hornless individuals in Qira black sheep.

Abbreviations

GWAS	Genome-wide association analysis
xp-EHH	Extended haplotype homozygosity
iHS	Integrated haplotype homozygosity score
Pi	Nucleotide diversity
W	Horned sheep group
A	Hornless sheep group
PCR	Polymerase Chain Reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11608-8.

Supplementary Material 1: S1 Table. iHS analysis of candidate gene values. In the table Start represents the start position of the gene on the chromosome and End represents the end position of the gene on the chromosome

Supplementary Material 2: S2 Table. XP-EHH analysis of candidate gene values. In the table Start represents the start position of the gene on the chromosome and the End represents the end position of the gene on the chromosome

Supplementary Material 3: S3 Table. Pi analysis of candidate gene values. In the table Start represents the start position of the gene on the chromosome and End represents the end position of the gene on the chromosome

Supplementary Material 4

Supplementary Material 5

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Author contributions

Data curation, Y.P.; Formal analysis, W.Z., X.L., X.Z. and X.B.; Funding acquisition, S. L.; Investigation, W.Z.; Methodology, W.Z. and Q.G.; Project administration, S.L.; Resources, W.Z. and X.B.; Software, W.Z. and R.Y.; Supervision, Z.H.; Visualization, W.Z. and X.L.; Writing– original draft, W.Z.; Writing– review & editing, W.Z., X. Z., L. Z. and Y.P.

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Data availability

The datasets analysed during the current study are available in the OMIX, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences repository, (https://ngdc.cncb.ac.cn/omix; GSA: OMIX008023).

Declarations

Ethics approval and consent to participate

This work was conducted in accordance with the standards set by the Ethics Committee of the College of Animal Science and Technology of Tarim University (protocol code: 2023039) and approved by the Ethics Committee of the College of Animal Science and Technology of Tarim University. Written informed consent was obtained from the owners for the participation of their animals in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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