BMC Genomics

RESEARCH

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Genome-based analysis of the genetic pattern of black sheep in Qira sheep

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Abstract

Objective Black wool can effectively prevent sheep from DNA damage as well as fungal infection, and can improve reproductive performance. In order to explore the candidate genes related to black wool formation in Qira sheep.

Methods We selected 123 adult healthy ewes with different coat colors in Qira sheep groups (black (B), brown (Y) and grey (G)) and extracted DNA from their venous blood to obtain Illumina Ovine SNP 50K chip data. Subsequently, our PCA, NJ-tree, and Admixture population structure analyses of the 3 wool color Qira sheep populations showed that the 3 middle wool color populations exhibited the same genetic traits. Fst, xp-EHH, iHS, and π were detected for selection signals, and the 5% SNPs loci positively selected from the analyses were annotated based on Shee-pOar_v4.0. The region of exon 1 of the *TYRP1* gene was further screened, amplified and sequenced through the DNA of the Qira sheep and associated with goodness-of-fit using the chi-square test.

Results The results showed that 71 SNPs associated with black wool traits, among which *TYRP1*, *PARD3* and *CDH2* genes were strongly associated with black wool production. Three mutations were detected in the exon 1 region of the *TYRP1* gene that were significantly associated with coat color variation inQira sheep (2:81,183,168, 2:81,183,281 and 2:81,183,284).

Conclusion In this paper, Qira sheep could not differentiate the genetic structure of this population by wool color, and obtained 71 SNPs related to black wool. Detection of mutation sites on the *TYRP1* gene affecting hair color change provides a basis for black sheep line selection as well as breed conservation.

Keywords Qira sheep, Illumina Ovine SNP 50K chip, Melanin, Fst, Pi, iHS, Xp-EHH

Background

The color of an animal's coat is an extremely important feature that affects its behavior and is decisive for its survival in the natural environment [1]. In modern animal farm animal breeding, adaptation and artificial selection have produced a wide variety of coat colors that have become the most distinctive features of different breeds [2]. Coat color can be used to distinguish among breeds [3]. There are 3 kinds of pigments in sheep's wool, melanin, brown pigment and yellow (red) pigment, which are mainly deposited in the cortical cells of wool fibers (the medullary cells also contain a small amount of pigment), the pigment type and content contained in the cortical cells of the sheep's wool fibers determines the color of the

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wool fibers, which further determines the type of wool color of the sheep's coat [4]. The results of many studies and numerous examples have revealed that wool color in sheep is controlled by compound alleles at multiple genetic loci and that black wool is effective against fungal infections [5]. At the same time, melanin color is also effective in preventing DNA damage from UV rays [6]. But the production of black wool is determined by melanin found in melanosomes in melanocytes [7] and can be categorized into true melanin and fucoxanthin. Eumelanin is exclusively responsible for the black to brown pigmentation of the skin and coat color, while fucoxanthin is responsible for the red to yellow coloration of mammalian coats [8].

Zhang et al. [9] showed that the ASIP gene was closely related to the diversity of wool color patterns in lambs. Lei et al. [10] identified candidate genes associated with hair follicle development and wool shedding using Illumina Ovine SNP 50K chip genotyping data from 27 wools of 27 breeds, determination of population differentiation index (Fst), nucleotide diversity (Pi), and extended haplotype homozygosity (xp-EHH) testing for selective characterization of wool and fine wool sheep. Kunene et al. [11] analyzed the inheritance of coat color in South African Engu cattle by Illumina Bovine HD (770K) chip and confirmed the role of the *MC1R* gene in the inheritance of coat color in Engu cattle. Guo et al. [12] found that the pigmentation gene *KITLG* gene showed a strong selective signature in Tibetan velvet goats by Fst analysis of goats. Wang et al. [13] Single nucleotide polymorphism (SNP) arrays, F-statistics (Fst), and heterozygous extended haplotype haploid test (xp-EHH) analyses of Affymetrix 600K HD information on Chinese and Kazakhstani sheep revealed six potential candidate genes associated with breed traits of the warped sheep. Adeniyi et al. [14] explored genetic diversity and selection traits using Illumina Ovine 50K SNP chip and Fst and xp-EHH analyses in Kosovo Balusha sheep.

Qira sheep is China's excellent lamb skin sheep, mainly distributed in the southwestern part of Xinjiang, the northern foothills of the Kunlun Mountains and the southern edge of the Taklamakan Desert. It belongs to the warm and extreme arid climate zone. It is mainly black, partly brown and grey. The genetics of its wool color is a necessary condition for the selection of Qira sheep for breeding improvement. We selected 123 Qira sheep with different wool colors under the same feeding conditions, analyzed the selection characteristics among 3groups based on their Illumina Ovine SNP 50K chip information, and screened the candidate genes related to the formation of black wool in Qira sheep based on the results of Fst, Pi, xp-EHH, and iHS, with the aim of providing a reference for the preservation breeding of the local breeds in Xinjiang.

Materilas and methods

Materilas

Xinjiang Jinken Aoqun Agriculture and Animal Husbandry Technology Co., Ltd. is located in Hetian, Xinjiang, and is one of the breeding bases of Qira sheep. The blood samples were collected from 123 healthy, 1–3 years old Qira sheep under the same housing management. According to the color portion of more than 90% of the coat area, the Qira sheep were studied and divided into three groups: black ($n=52$, B), brown ($n=30$, Y), and grey ($n=41$, G) (Fig. 1).

Data processing

Genotyping was performed based on Illumina's SNPline genotyping platform from Beijing Compassion Biotechnology Co. Ltd. and a dataset consisting of data from 123 individuals with individual SNPs was obtained. Quality control was performed using Plink v1.90 [15], with maximum missing rate (max-missing) < 10%, i.e., SNP loci with > 90% detection rate, and minimum allele frequency (MAF) > 0.01 (*P*-value of Hardy–Weinberg equilibrium test > 10^-6 -hwe); the data were analyzed using the –min-alleles 2 –max-alleles 2 method. Alleles 2 to remove multiple alleles and allelic deletions. The final 52,780 SNPs were available for subsequent analysis.

Population structure

PCA analysis

Data on SNPs after quality control were analyzed by PCA using PLINK software, a principal component analysis that identifies major components representing population structure based on genetic correlations between individuals. The number of reductions in the top-ranked PCs was calculated by projecting the samples onto the space spanned by the eigenvectors of the sample covariance matrix and selecting the eigenvectors that contained the largest contribution of variation in the samples [16], using 'plink -pca 3'. The data results were visualized using R.

NJ-tree

The P-distance matrix was calculated using the VCF2D is software (https://github.com/BGI-shenzhen/VCF2Dis), and the NJ-tree was constructed using the ATCG online program [17] for the matrix results, which were plotted using the web tool iTOL 16.

Admixture

The 3 coat color groups of Qira sheep were analyzed using Admixture [18], and the data after quality

Fig. 1 Schematic diagram of wool color classification criteria of Qira sheep

control were trimmed for LD in a sliding window of 50 SNPs, and Admixture analysis was performed for K-values between 2–3, and the results were visualized and presented using Prel scripts.

Selective signatures

Different biological analysis tools have different results, but no single method can completely detect all biological results in a genome-wide selective scanning analysis [19]. In order to capture genomic selection signals more accurately, multiple methods are usually required [19], and for this reason we chose four complementary statistical analysis tests: Fst (fixation index), xp-EHH (extended haplotype homozygosity), iHS (integrated haplotype homozygosity score) and Pi (nucleotide diversity). This study analyzes Fst, xp-EHH between the black and non-black groups, and Pi, iHS for the black group alone. We refer to the results of the analysis between the black wool population (B) and the brown wool population (Y) of Qira sheep as BY, and the analysis between the black wool population (B) and the grey wool population (G) of Qira sheep as BG.

Fst analysis

Genetic diversity between populations or subspecies can be studied by Fst analysis. The Fst [20] between black and non-black populations was calculated using VCFTOOLS to measure the genetic differences between populations, which is used to determine the degree of genetic differentiation between populations. Higher Fst values imply greater genetic differentiation between populations, and vice versa. Selection traits between black, brown and grey populations within breeds were analyzed with a sliding window set at 50,000 bp and a step size of 12,500 bp, and the Fst analysis was calculated as:

$$F_{st} = \frac{MSP - MSG}{MSP + (N_c - 1)MSG}$$

MSG is the mean square of the error of the detected intra-population loci, MSP is the mean square of the average variance of the loci between the tested populations, and NC denotes the corrected mean between the overall sample sizes.

Pi analysis

Nucleotide diversity, denoted as π, is an important concept in molecular genetics used to quantify genetic diversity within or between populations. The calculation of nucleotide diversity is based on the average of the same site base differences on the DNA sequences of multiple samples obtained from a population, reflecting the average proportion of base differences between the DNA sequences of different individuals within a population, and thus is widely used to characterize the level of genetic diversity of a population. Black population chip data of Qira sheep were analyzed for population nucleic acid diversity using VCFTOOLS. We set the sliding window of pi analysis to 500kb set the step size to 50kb, and the SNP loci with 5% of the final pi value were considered as significant candidates [21] for analyzing polymorphisms in the black wool population of Qira sheep.

Xp-EHH

Xp-EHH was used to compare selection signals between populations, we used Selscan to do heterozygous extended haplotype purity test (xp-EHH) [22] on 50K SNP chip data of Qira sheep to compare. The haplotypes of the black wool population of Qira sheep with those of the non-black population, the top 1% loci were extracted using –cutoff 0.01, and the xp-EHH statistic was calculated using the formula:

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

where unXPEHH is set:

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

iESpop1 integrates EHH genetic distance statistics, and iESpop2 integrates genetic distances from EHH statistics of the black population.

IHS

The IHS is used to detect selection signals within individual populations, we used rehh to measure the Qira Sheep Black Herd individually for the Integrated Haplotype Purity Score (iHS) [23], using individual marker loci to replace the core haplotypes in the EHH statistic, defining them as core loci. And their ratios were calculated to select the signal detection statistic used to complement the Fst analysis, calculated as:

$$iHS = \frac{uniHS - mean(uniHS\ |P_s)}{sd\ (uniHS\ |P_s)}$$

where 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.

Candidate gene detection and annotation

For each scan result use R to visualize and analyze the results. For each scan, we took the top 5% SNP sites for Fst, xp-EHH, and iHS values, and only the last 5% SNP sites for θπ ratio analysis were taken as positively selected sites to identify the selected regions in the genome, and all the obtained SNPs were annotated based on Oar_v4.0 (https://www.sheephapmap.org/). 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/) [24] and Kyoto Encyclopedia of Genes and Genomes (KEGG, Kyoto Encyclopedia of Genes and Genomes) [25] were analyzed. 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 the FinePure Blood/Cell/Tissue Genomic DNA Extraction Kit (spin column type) (Jifan Biotechnology Co., Ltd., Beijing, China) according to the kit manufacturer's instructions, DNA quality was tested using 1.5% agarose gel electrophoresis dissolved in 1X TBE buffer, and visualized using a fully automated gel imager (Hangzhou Haipei Instrument Co., Ltd., Hangzhou, China). A nucleic acid protein detector (Shenzhen Enke Biotechnology Co., Ltd., Shenzhen, China) was used to detect the concentration of the extracted DNA.

Polymerase chain reaction

The *TYRP1* exon 1 gene was amplified using PCR (Life Pro Thermal Cycler, Hangzhou, China) and the optimal annealing temperature was determined using gradient thermal gradient PCR. A total of 50 μl of DNA 2 ul at 30 ~ 100 ng/ul, 2 ul with 10 uM forward and reverse primers, and 2×Taq PCR mix (with dye/blue) 25 ul were used. The following thermal cycling conditions are used: denaturation at 94℃ for 5 min, then 30 s at 94℃,

then annealing at 59℃ for 30 s, extension at 72 ℃ for 1 min, and finally 10 min at 72℃ (Table 1) [26]. PCR product quality was detected using 1X TAE 1.5% agarose gel electrophoresis.

Sequencing procedure

After the PCR products were prepared and purified, the PCR products were tested in one direction using the Sanger sequencing method at Sangon Bioengineering (Shanghai) Co., Ltd., and the sequencing results were compared using Chroms software. Allele frequencies were calculated using direct counting.

Results

Analysis of the population structure

Results of PCA analysis

PCA analysis of the 3 clusters revealed almost no differences between different wool colors from the same group, with all eigenvalues essentially ≤ 1 (Fig. 2(a)), and a slight difference between the peak contribution between the grey wool group and the other two groups can be seen through the vertical and horizontal axes.

NJ-tree

In addition, the phylogenetic tree (Fig. 2(b)) shows that there are no major genetic differences between the various wool colors of Qira sheep, showing a mixed distribution. Some of the grey wool and black wool color groups showed aggregation, and the brown wool groups were evenly distributed among the groups.

Admixture result

An admixture analysis with K = 2–3 was attempted for the 3 groups of Qira sheep, and it was found that the lowest error values were detected when K = 3, but there was no clear separation between the individual color groups (Fig. 2(c)).

Selection of signal analysis results

Fst

By analyzing the genetic differentiation among Qira sheep groups with different wool colors, the study used the genomes of Qira sheep black wool depopulation group as the reference group, and the brown and grey wool groups as the experimental groups for the analysis of selection signals. The results of the Fst analysis of the Qira sheep showed that the degree of genetic differentiation indicated by the BY and BG analyses was similar, but the degree of genetic differentiation of the BY group was relatively high. Significant loci of BG analysis were mainly distributed on chromosomes 3, 4, 10, 13, and 17 (Fig. 3(a)), and the significant loci of BY analysis were mainly distributed on chromosomes 2, 4,14, and 16 (Fig. 3(b)). The top 5% of Fst values were considered as the positive selection region, and the genetic differentiation coefficient was greater than 0.05. The top 5% of Fst values for the black and grey populations were distributed between 0.0436708 and 0.321588, with a mean value of 0.063. The 3219 SNPs were obtained. the distribution of Fst values between the brown and black populations ranged from 0.0265953 to 0.232084 with a mean value of 0.043. The 3214 SNPs were obtained. Their valid SNP loci were annotated, and 3219 valid genes and 3214 valid genes were annotated in the two groups analyzed, respectively. According to the results of intersection statistical analysis of VEEN (Fig. 3(c)), a total of 1175 shared genes were detected in the selected region, of which 1086 genes were recognized by DAVID software. In addition, the results showed that 39 KEGG pathways were enriched as well as 102 GO terms were enriched ($p < 0.05$) (Supplementary Table S1). Among the relevant GO terms, we found the melanocyte differentiation pathway (GO:0030318), in which *MEF2C, TYRP1, GLI3, ETBR* genes were shown.

Pi result

Nucleotide polymorphism analysis of the black wool population of Qira sheep was found to be more significant on chromosomes 1, 3, 10, 22 and 27 (Fig. 4). The last 5% SNP loci of this analysis were taken as selected loci for annotation and 2412 SNPs were selected, yielding a total of 2412 valid genes. Of these, 2231 genes were softly recognized by DAVID and were enriched to 72 KEGG pathways and 298 GO terms ($p < 0.05$) (Supplementary Table S2). Among the relevant pathways, *MEF2C, MREG, EDN3, KIT, TYRP1, BCL2, MITF, GLI3,* and *ETBR* genes were shown to be on the melanocyte differentiation pathway (GO:0030318).

Xp-EHH result

The genomic region of Qira sheep wool color was investigated through the differences in extended haplotype purity among Qira sheep groups with different wool colors, and the black wool group was used as the reference population, and the brown and grey groups were used as the experimental populations for the selection signal analysis. The xp-EHH analysis of different phenotypes of Qira sheep revealed higher frequencies of favorable alleles in the BY analysis cohort, and the genetic diversity analysis was more significant on chromosomes 1, 2, 21, and 23 for the BY cohort (Fig. 5(a)) and on chromosomes 3, 4, 5, 13, 15, and 16 for the BG cohort analysis (Fig. 5(b)). The top 5% of SNP loci from both groups analyzed were taken as positive selection loci for annotation; 1603 SNPs were selected in the brown group and 1587 SNPs were

Table 1 Major candidate genes

Values for each analysis of the total intersecting genes							
chrom	**SNP name**	**Postion/bp**	**Fst Value**	**xp-EHH Value**	**PI Value**	**iHS Value**	**Gene symbol**
1	OAR1_13033760.1	3275	0.0487154	0.109669	0.0000309091	0.574924	*UTP11L,RRAGC*
1	OAR1_171959158.1	25,227	0.04731	0.101633	0.0000351199	0.591559	*NSUN3*
1	OAR1_13042261.1	3275	0.0487154	0.109669	0.0000309091	0.574921	*UTP11L,RRAGC*
2	OAR2_85779058.1	52,387	0.0817096	0.112629	0.0000331906	0.524973	***TYRP1***
2	OAR2_202228555.1	63,675	0.062447	0.117695	0.0000183742	0.570948	*CNTNAP5*
2	OAR2_105835095_X.1	53,610	0.0758777	0.0985519	0.0000239805	0.565734	*LINGO2*
2	OAR2_139245831.1	57,862	0.0700108	0.103176	0.000025273	0.65308	*OSBPL6,RBM45*
2	OAR2_238212834.1	69,170	0.0469031	0.0853471	0.0000199025	0.594302	*SERPINE2,FAM124B*
3	OAR3_170628446.1	103,456	0.050841	0.107147	0.0000177537	0.638628	*FAM19A2,SLC16A7*
3	OAR3_59402026.1	86,683	0.0985777	0.148821	0.000033112	0.533681	*SUCLG1*
3	OAR3_227674946.1	114,111	0.0630127	0.0880211	0.0000176887	0.700455	*CCND2,PARP11*
3	OAR3_227674946.1	114,102	0.0630127	0.0880211	0.0000256198	0.700455	*CCND2*
3	OAR3_59339649.1	86,683	0.0985777	0.148821	0.000033112	0.533681	*CTNNA2,SUCLG1*
4	OAR4_15046925.1	120,326	0.0957514	0.0866423	0.000020859	0.513963	*TAC1*
4	OAR4_19938568.1	120,584	0.0476295	0.100748	0.0000179759	0.695198	*PHF14,THSD7A*
4	OAR4_56538053.1	124,190	0.0441561	0.122422	0.0000250345	0.585644	*TFEC,MDFIC*
4	OAR4_19365053.1	120,525	0.0710087	0.137	0.0000182767	0.600264	*NXPH1*
6	OAR6_61276064.1	159,318	0.0616372	0.101428	0.0000252594	0.64475	*DTHD1*
6	OAR6_51969295.1	159,215	0.0615088	0.118127	0.0000200677	0.586205	*STIM2*
7	OAR7_43964022.1	176,616	0.114183	0.0934832	0.0000270912	0.644868	*LRR1*
7	OAR7_105194400.1	185,812	0.0456056	0.103503	0.0000284108	0.725849	*GALC*
8	OAR8_71441594_X.1	2,450,666	0.0445234	0.0916673	0.0000261265	0.785571	*CITED2,NMBR*
8	OAR8_71441594_X.1	192,844	0.0445234	0.0916673	0.0000261265	0.785571	*CITED2,NMBR*
8	OAR8_17015207.1	187,861	0.0503064	0.173811	0.000039244	0.825345	*TRDN,CLVS2*
9	OAR9_2098219.1	196,306	0.0531923	0.16075	0.0000282834	0.702268	*RIMS1*
9	OAR9_6698498.1	196,664	0.0445281	0.107961	0.00000657093	0.585131	*PRKAR1A*
10	OAR10_91326471_X.1	213,170	0.0841833	0.169319	0.0000224143	0.500972	*MYO16*
10	OAR10_49791280.1	210,851	0.0567908	0.144945	0.0000275681	0.783978	*KLF5,KLF12*
10	OAR10_53546970.1	211,045	0.0636752	0.103662	0.0000320688	0.78878	*KCTD12*
10	OAR10_48194802.1	210,714	0.164751	0.140619	0.0000339791	0.665633	*DACH1*
10	OAR10_62745928.1	211,482	0.0827917	0.101712	0.000029202	0.542474	*SLITRK1*
12	OAR12_62830594.1	247,897	0.0589756	0.0899968	0.0000301748	0.886186	*ASTN1,BRINP2*
12	OAR12_73766431.1	249,844	0.0483945	0.100198	0.0000299743	0.623256	*CENPF*
13	OAR13_20943553.1	253,910	0.0440328	0.181943	0.0000207397	0.575289	*PARD3,NRP1*
15	OAR15_77451211.1	298,919	0.153398	0.122069	0.000024284	0.828211	*LRRC4C*
15	OAR15_80616593.1	299,353	0.0533936	0.113271	0.000014624	0.728464	*GYLTL1B,PHF21A*
16	OAR16_30048572.1	304,998	0.119315	0.107378	0.0000263433	0.552281	*PELO,ISL1*
16	OAR16_30048572.1	304,998	0.11931	0.107378	0.0000263433	0.552281	*PELO,ISL1*
16	OAR16_56828558.1	307,147	0.0669117	0.0916827	0.0000278472	0.822235	*CDH12,FTL*
16	OAR16_14425857.1	303,576	0.0440164	0.119159	0.0000343124	0.645323	*MAST4,SREK1*
16	OAR16_31959917.1	305,085	0.0602716	0.103197	0.0000191627	0.690585	*HCN1*
17	OAR17_35667529.1	310,950	0.066421	0.109718	0.0000100393	0.598074	*FAT4*
17	OAR17_65144494.1	315,741	0.0645432	0.1126	0.0000196179	0.507854	*MED13L,TBX3*
17	OAR17_40466743.1	311,323	0.0743566	0.106884	0.0000380165	0.536769	*FstL5*
17	OAR17_65144494.1	315,741	0.0645432	0.1126	0.0000264029	0.507854	*MED13L,TBX3*
17	OAR17_33858663.1	310,941	0.0979368	0.0955128	0.0000278255	0.509425	*INTU*
17	OAR17_53899478.1	312,808	0.0748042	0.154523	0.0000192982	0.710431	*TMEM132C*
18	OAR18_18094841.1	322,055	0.118674	0.139239	0.0000758054	0.65751	*AGBL1*

Table 1 (continued)

Values for each analysis of the total intersecting genes							
chrom	**SNP name**	**Postion/bp**	**Fst Value**	**xp-EHH Value**	**PI Value**	**iHS Value**	**Gene symbol**
18	OAR18_41453420.1	326,195	0.0855244	0.137149	0.0000185612	0.629766	*FOXG1,PRKD1*
18	OAR18_37228030.1	326,165	0.100254	0.116842	0.0000311855	0.998734	*STXBP6,NOVA1*
18	OAR18_13826286.1	321,888	0.0652288	0.0948501	0.0000280206	0.962871	*ST8SIA2,SLCO3A1*
18	OAR18_15029766.1	321,910	0.0652288	0.0948501	0.0000280206	0.705537	*SV2B*
18	OAR18_7399886.1	321,676	0.0961646	0.108525	0.0000369869	0.595985	*FAM169B,ARRDC4*
18	OAR18_12976100.1	321,748	0.0690067	0.108846	0.0000282753	0.658714	*MCTP2,RGMA*
18	OAR18_41453420.1	326,195	0.106241	0.137149	0.0000185612	0.629766	*FOXG1,PRKD1*
18	OAR18_12976100.1	321,748	0.0690067	0.093598	0.0000282753	0.658714	*MCTP2,RGMA*
19	OAR19_49422424.1	336,647	0.0712599	0.0893823	0.0000282157	0.59763	*CACNA2D3*
19	OAR19_43203433.1	335,758	0.0767096	0.104079	0.0000263677	0.544493	*RPL17*
20	OAR20_15759478.1	346,275	0.0570854	0.113584	0.0000305595	0.500417	*LRFN2,UNC5CL*
22	OAR22_53294394.1	373,937	0.0965383	0.160199	0.00003693	0.546223	*MKI67,MGMT*
22	OAR22_6749661.1	366,458	0.0667504	0.113515	0.0000287251	0.832573	*PCDH15*
22	OAR22_28593912.1	371,024	0.0904602	0.132173	0.0000292996	0.603851	*CCDC147,SORCS3*
22	OAR22_53294394.1	373,937	0.0965383	0.117296	0.00003693	0.546223	*MKI67,MGMT*
22	OAR22_6749661.1	366,458	0.04731	0.113515	0.0000223466	0.601225	*PCDH15,SYCE1*
22	OAR22_33667556.1	371,156	0.112874	0.132173	0.0000285408	0.771684	*SORCS1,XPNPEP1*
23	OAR23_30351189.1	376,466	0.0629906	0.112886	0.0000240618	0.764535	*DSC3,CDH2*
23	OAR23_30351189.1	376,466	0.0629906	0.112886	0.0000194608	0.764535	*DSC3,CDH2*
23	OAR23_54785906.1	380,060	0.062278	0.145152	0.0000263894	0.722616	*AP3S1,DCC*
24	OAR24_8063846.1	384,977	0.0506308	0.152939	0.0000260507	0.544866	*RBFOX1*
25	OAR25_10674440.1	397,325	0.0689671	0.105289	0.0000283945	0.711577	*RYR2,ZP4*
25	OAR25_9968434.1	397,242	0.0882045	0.1233	0.0000201192	0.610256	*RYR2*

selected in the grey group. 1603 valid genes as well as 1587 valid genes were obtained, respectively, and a total of 521 genes were analyzed in the two groups according to VEEN statistics (Fig. 5(c)). Among them, 498 genes were identified by DAVID software and the results showed that 18 KEGG signaling pathways were enriched and 48 GO terms were enriched ($p < 0.05$) (Supplementary Table S3). One of the pathways associated with melanoma (oas05218) is shown.

IHS result

Gene signaling selection (iHS) based on the haplotype of the black population of Qira sheep was found to be more significant on chromosomes 2, 5, 7, 18, and 19 (Fig. 6), and the top 5% of the loci were taken as the selected loci for annotation, which resulted in 18,079 SNPs annotated to 2,271 genes, of which 1,893 genes were identified by the DAVID software, and enriched to 169 GO pathways and 36 KEGG pathways ($P < 0.05$) (Supplementary Table S4). The genes *FZD1, EDN1, CREBBP, FZD4, WNT5A, TYRP1, WNT8B, MITF, ADCY1, ADCY8, GNAI1, ADCY9, WNT11, PLCB4, WNT2,* and *LEF-1* were shown to be in the melanin production pathway (oas04916).

Intersection analysis

After annotating the results of each group, the results were processed through the VEEN online website (https://bioinformatics.psb.ugent.be/webtools/Venn/) and found that there were 213 valid genes in the overlapping candidate regions between the black group and the brown and grey groups of Qira sheep. Among these genes, there were 613 intersecting genes in the overlapping candidate regions of haplotype analysis and pi analysis of the black population. To further identify the candidate genes for the black wool of Qira sheep, we further analyzed the overlapping candidate regions of the genes of the four analyses and found 71 intersecting genes (Fig. 7, Table 2, Supplementary Table S5). Among these 71 genes we identified the *TYRP1, PARD3*, and *CDH2* genes, which have been reported to be associated with melanin, by comparison with the Color Gene Library (http://www.ifpcs.org/colorgenes/), and we also found the presence of these genes to be associated with hair growth (*DSC3, INTU*), reproduction (*ARRDC4, PRKD1,CCND2*), fleshy growth and adipogenesis

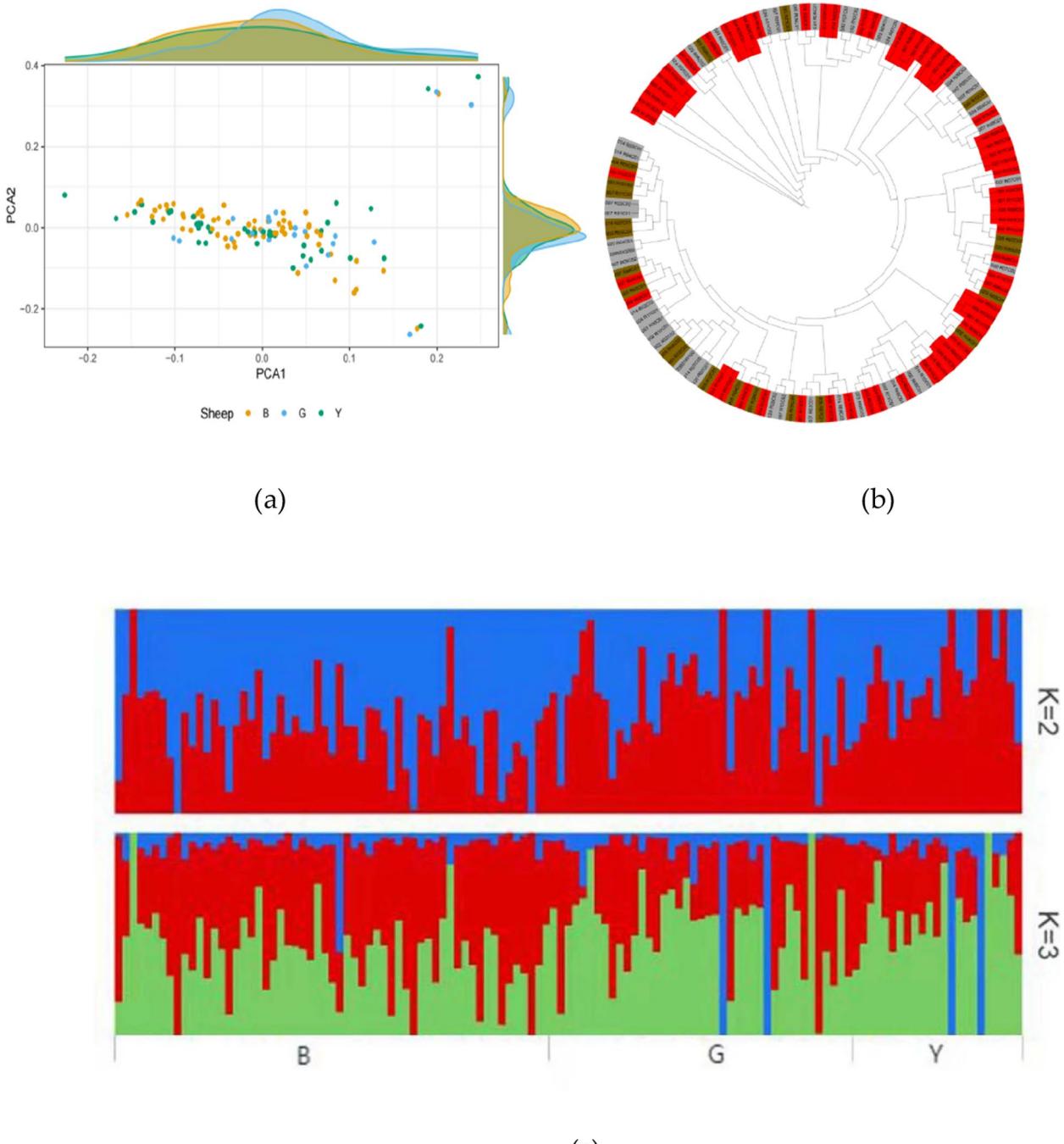

Fig. 2 Results of genetic structure analysis. **a** PCA chart.Yellow dotted B represents the black wool group of Qira sheep, blue dotted G represents the grey group of Qira sheep, and green dotted Y represents the brown group of Qira sheep. **b** Phylogenetic tree of different wool color groups in Qira sheep. Brown stands for the brown wool group of Qira sheep Grey stands for the grey wool group of Qira sheep Black stands for the black wool group of Qira sheep. **c** Genetic mixing analysis of different coat colors of black sheep in Qira. B represents the black wool group of Qira sheep, G represents the grey wool group of Qira sheep, and Y represents the brown group of Qira sheep

(*FSTL5, MCTP2, MDFIC, RPL17, SORCS1, SORCS3, KLF12, PCDH15, THSD7A, NXPH1*), follicular and embryonic development (*BRINP2, CDH12, CENPF,*

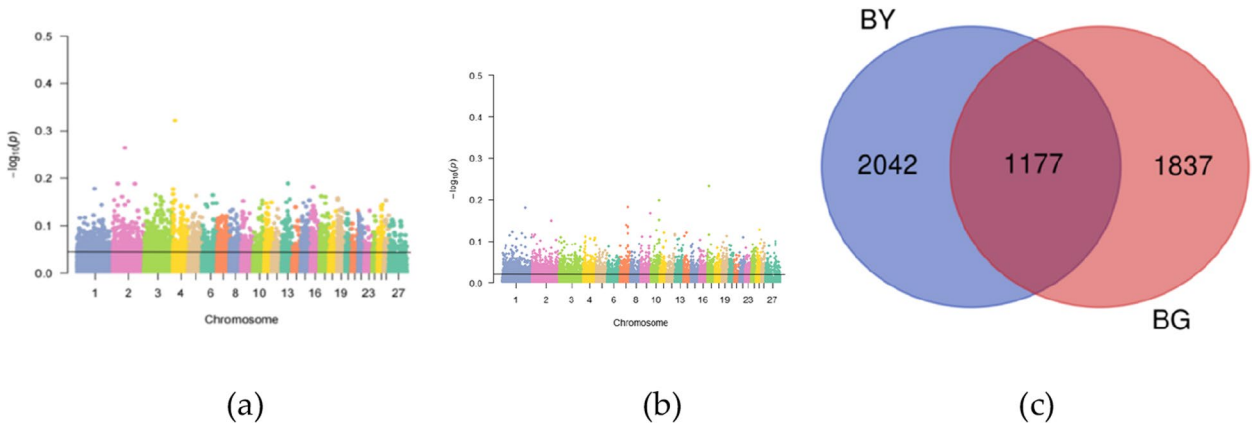

Fig. 3 Fst analysis. **a** BG Fst analyzes Manhattan chart, Analysis of genetic differentiation between black and grey populations of BG-representative Qira sheep. The black line in the graph represents the threshold line for the top 5% of choices. **b** BY Fst Analytics Manhattan Chart, Analysis of genetic differentiation between black and brown wool populations of Qira sheep represented by BY. The black line in the graph represents the threshold line for the top 5% of choices. **c** VEEN diagram for Fst, BY represents the genes annotated and identified by the genetic differentiation analysis of the black and brown wool populations of Qira sheep, with 3219 valid genes, and BG represents the genes annotated and identified by the genetic differentiation analysis of the black and grey populations of Qira sheep, with 3014 valid genes, where the overlapping portion is the common genes analyzed in the two groups, with 1177 genes

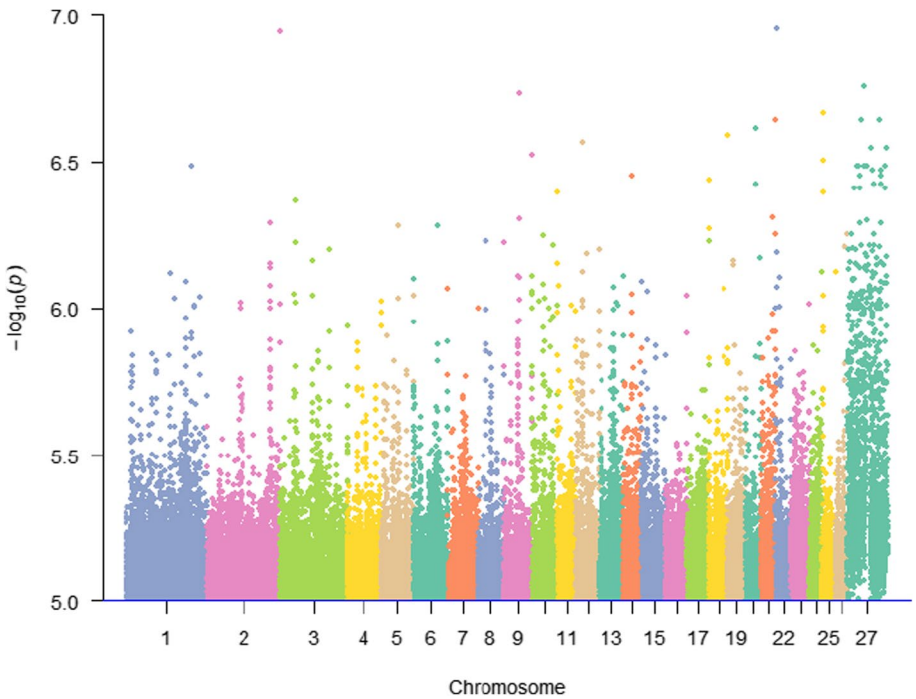

Fig. 4 Manhattan plot of pi analysis. The pi analysis of black wool population of Qira sheep

DTHD1, PHF21A, RIMS1, SYCE1, TAC1, CCND2, ZP4, CACNA2D3), genes related to mammary gland development and milk production (*LRRC4C, MYO16, TMEM132C, SLCO3A1, OSBPL6*), and litter size in sheep (*DACH1*).

DNA extraction 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 the nucleic acid detector, it is found that the integrity of DNA meets the experimental requirements, and the OD260 nm/OD280 nm is in the range of 1.9 ~ 2.0.

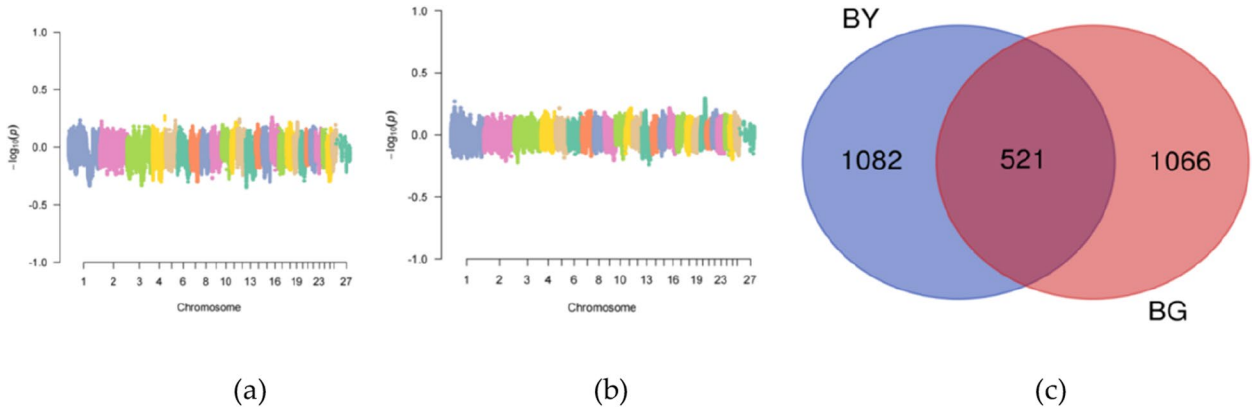

Fig. 5 Xp-EHH analysis. **a** BY group XPHH analyzes Manhattan charts, Analysis of haplotype selection signals between the black wool group and the brown group of Qira sheep represented by BY. **b** XP-EHH analysis of BG groups Manhattan charts, BG representative measured haplotype selection signal analysis of black wool populations versus grey wool populations in black sheep. **c** XP-EHH intersection analysis plot, The blue part BY represents the genes identified by the haplotype selection signal analysis and annotation of the black wool group and the brown group of Qira sheep, with 1603 genes, and the pink part BG represents the genes identified by the haplotype selection signal analysis and annotation of the black wool group and the grey wool group of the measured black sheep, with 1587 genes, and the overlapping part is the 521 genes shared by the two groups of analyzed genes

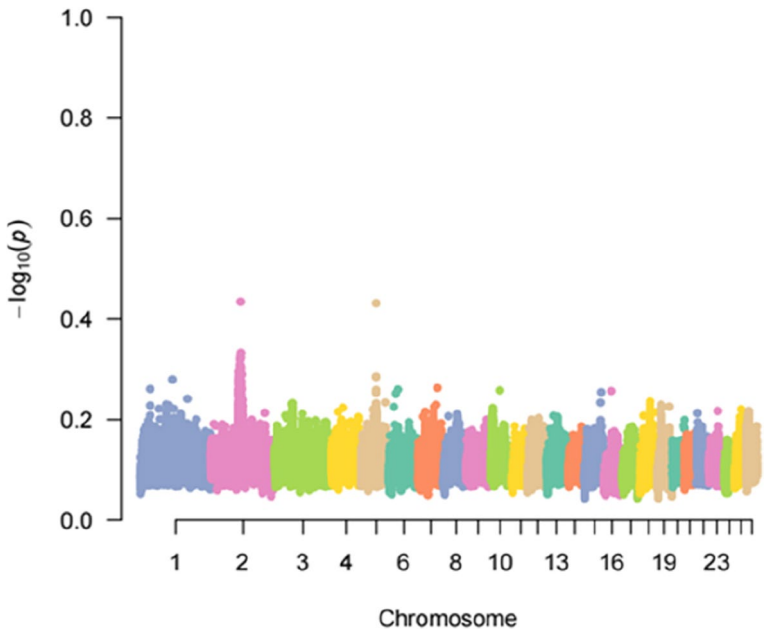

Fig. 6 iHS Manhattan Chart. iHS analysis of the black population of Qira sheep

Results of PCR experiments

The results of the amplification of the exon 1 region of the *TYRP1* gene of the Qira sheep are shown in the Fig. 9, and the amplification products of the PCR products are consistent with the expected size of the amplified fragments, which can be used for subsequent experiments.

Mutation site detection

Three mutations were observed in the 470 bp PCR product generated from the exon 1 region of the *TYRP1* gene (Fig. 10), the first mutation site 2:81,183,168 changed serine to cysteine, the second mutation site 2:81,183,281 changed valine to alanine and the third mutation site 2:81,183,284 changed isoleucine to threonine. The results

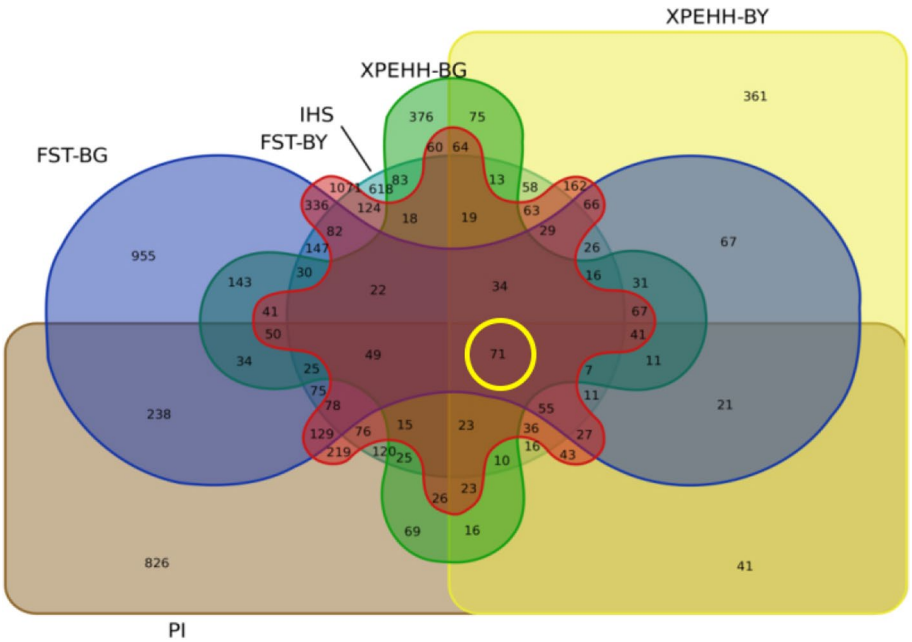

Fig. 7 Intersection plot of all genes in the population analysis of Qira sheep. The yellow region (xp-EHH-BY) in the Fig. represents 1063 valid genes annotated in the valid candidate region of the xp-EHH analysis between the black and brown populations of Qira sheep; the brown region (Pi) represents 2412 valid genes annotated in the pi analysis of the black wool population of Qira sheep; the dark blue region (FST-BG) represents 3014 valid genes annotated in the valid selection region of the Fst analysis between the black and grey population of Qira sheep; and the red region (FST-BY) represents 3014 valid genes annotated in the valid selection region of the Fst analysis between the black and brown population of Qira sheep. The dark blue region (FST-BG) represents the 3014 active genes annotated in the active selection region of the Fst analysis between the black and grey populations of Qira sheep; the red region (FST-BY) represents the 3219 active genes annotated in the active selection region of the Fst analysis between the black and brown populations of Qira sheep; and the light blue circular region (iHS) represents the 2271 active genes annotated in the active selection region of the iHS analysis of the black wool population of Qira sheep. 2271 effective genes. The portion marked by the yellow circle represents the 71 genes common to the above analyses

Table 2 Study gene with forward and reverse primers, product length, and optimal annealing temperature

Region	Primers	Product Length (bp)	Annealing Temperature	GenBank
TYRP1 Exon 1	F: TGCCTCCTTCTCTACACAAA R: ATCCATTCAGTCTCCGCTTA	470	59℃	EU760771.1

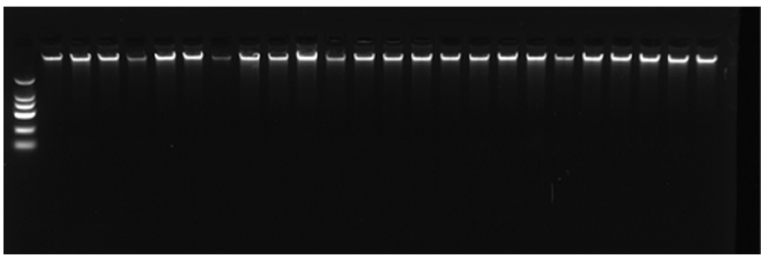

Fig. 8 Results of DNA extraction from blood samples

of the chi-square test (Table 3) showed that mutations at these three loci showed significant correlation ($P < 0.05$) with the production of different coat colors in Qira sheep.

Discussion

This study explores the coat color selection traits of the breed and candidate genes associated with black coat

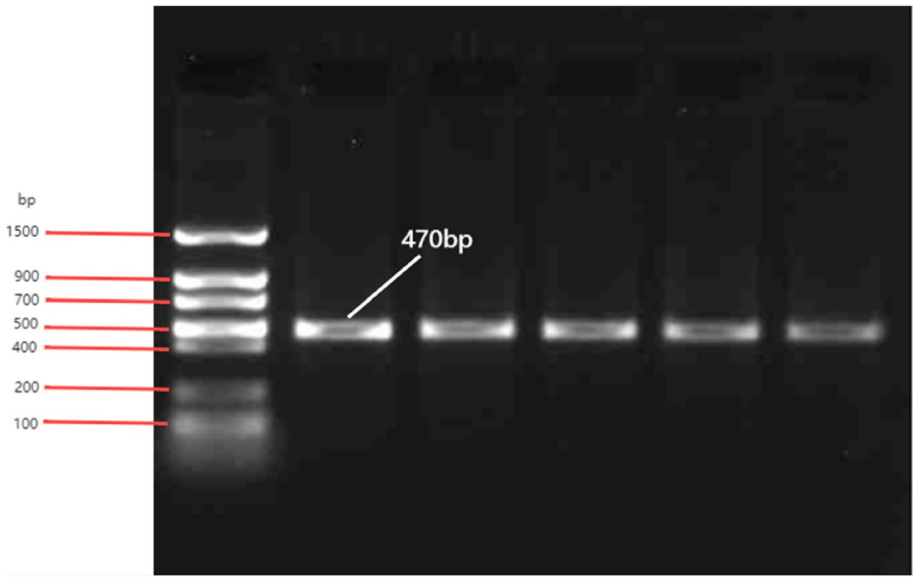

Fig. 9 PCR amplification results

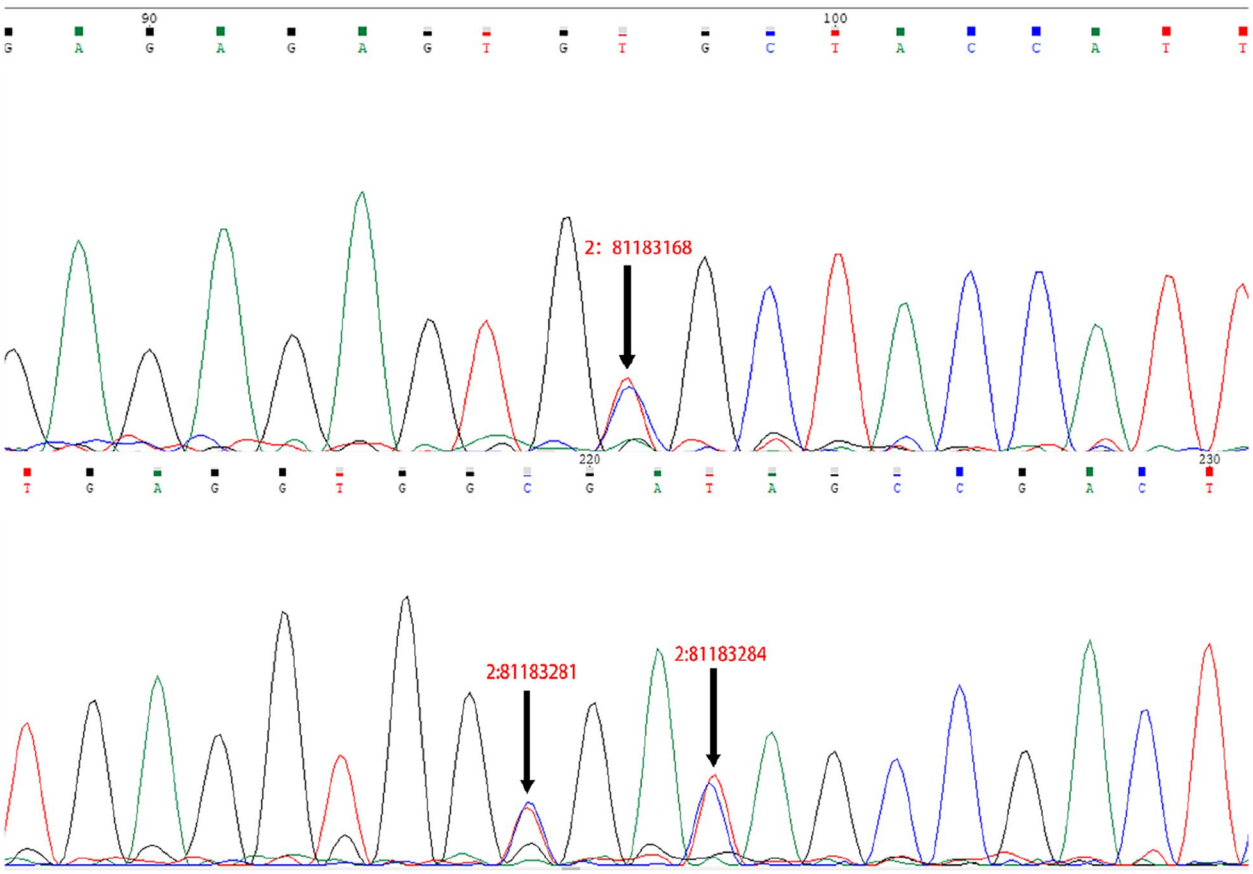

Fig. 10 TYRP1 exon 1 region gene detection mutation sites

Table 3 Summary of detected mutations within targeted genomic loci

Region	Mutation Locus	Mutant Nucleotides	Amino Acid Alteration	Proportion of black groups	Proportion of non-black groups
TYRP1 exon 1	2:81,183,168	C/T	Serine to Cysteine	34%	66%
	2:81,183,281	T/C	Valine to alanine	27%	73%
	2:81,183,284	T/C	Isoleucine to threonine	29%	71%

color selection based on Illumina Ovine SNP 50K chip data from 123 Qira sheep of 3 coat colors. We used PCA to assess their selection traits for differences in coat color. This method was used to assign them to groups using genotypic markers. When categorization is not obvious and animals of the same breed tend to be located in close proximity in PCA maps [27]. The PCA analysis of the 3 coat colors of Qira sheep revealed that the experimental Qira sheep were all in the same genetic background, and the genetic differences in their coat colors were not obvious, and further construction of the NJ-tree of individuals in different coat color groups revealed that all individuals were under the same genetic branch, and that the individuals of each coat color were evenly distributed on each branch. In the admixture analysis of the 3 groups, the lowest error value was detected when K = 3, and there was no genetic differentiation among the 3 groups. The above results showed that the genetic characteristics between different coat colors of Qira black sheep were the same, and they were simple in genetic background, which indicated that it was not possible to classify Qira sheep from coat color.

The results of the Fst analysis showed that there was no genetic differentiation between the 3 coat color groups of Qira sheep, and the pathway enriched for the same genes in the BY and BG populations showed the melanocyte differentiation pathway, where *MEF2C, MREG, EDN3, KIT, TYRP1, BCL2, MITF, GLI3*, associated with melanin formation, was shown *ETBR* genes. Nucleotide polymorphisms in populations could also be resolved on the basis of Illumina Ovine SNP 50K chip data. Pi analysis showed that the distribution of nucleic acid diversity differences in the black wool population was more homogeneous on each chromosome, while there were more significant loci on chromosome 2. Among the significantly selected loci, the genes *MEF2C, MREG, EDN3, KIT, TYRP1, BCL2, MITF, GLI3,* and *ETBR* were shown to be on the melanocyte differentiation pathway (GO:0030318). Among the Xp-EHH analysis selected loci, we identified the KEGG signaling pathway associated with melanoma formation, on which *CDK6, FGF9, PDGFD, PDGFC, FGF-10, GADD45G* genes were shown. iHS analysis showed strong selection signals on chromosomes 2 and 5. *ZD1, EDN1, CREBBP, FZD4, WNT5A, TYRP1, WNT8B, MITF, ADCY1, ADCY8, GNAI1, ADCY9, WNT11, PLCB4, WNT2, LEF-1* were displayed in the melanogenesis pathway. *TYRP1* genes were displayed in pathways related to melanogenesis.

Melanogenesis is a very complex process that involves the development, survival and differentiation of melanocytes [28]. The process of melanin synthesis has been extensively studied, and the amino acid tyrosine is thought to be the starting material for melanin synthesis [29]. Genes encoding tyrosinase control tyrosine-based melanin anabolism and facilitate the conversion of tyrosine to melanin [30] the Wnt signaling pathway, on the other hand, would be involved in pigmentation and melanin formation. Mammalian coat color phenotype is controlled by melanin secreted by melanocytes, melanin is divided into true melanin and fucoxanthin, both of which accumulate into melanosomes, and ultimately the number and proportion of their distributions contribute to the diversity of mammalian coat color [31], genetic mutations under certain circumstances induce the transformation of true melanin to fucoxanthin and the graying of the animal's coat color is mainly associated with the loss of melanocytes associated [32]. Animal coat color changes are mainly regulated by genetic and environmental factors, and some findings suggest that the activity as well as the expression of *TYRP1* is related [33]. Beraldi et al. [34] developed a complete linkage map to identify loci responsible for determining several phenotypic traits, including hair color, which suggests that *TYRP1* is a strong candidate gene for influencing wool color in sheep.

In order to accurately screen candidate genes related to black wool formation in Qira sheep, we annotated the overlapping regions of the four signal selection signals in this study, and annotated 71 shared candidate genes (Supplementary Table S5).

Tyrosinase-related protein 1 (*TYRP1*) is located on chromosome 2 in sheep, with physical location NC_056055.1 (81,182,553- 81,201,184), and has been reported in numerous reports confirming its significant correlation with pigmentation, it is an important enzyme that affects the quality and quantity of melanin production [27]. Beraldi et al., (2006) et al. identified the *TYRP1* gene as a strong candidate for influencing wool

coloration in sheep by analyzing the complete linkage mapping of sheep.

In this study, PCR amplification of exon 1 of the TYRP1 gene from Qiras sheep showed that all three mutation sites were significantly associated with causing changes in coat color in Qira sheep, with a C to T mutation at 2:81,183,168 causing the amino acid edited to produce a serine to cysteine change, and 34% of the individuals with black wool and 34% of the individuals with non-black wool were observed at this mutation site. individuals accounted for 66%. The presence of a T to C mutation at 2:81,183,281 caused a change in the edited amino acid from valine to alanine, which was observed in 27% of black wool individuals and 73% of non-black wool individuals at this mutation site. A T to C mutation was present at 2:81,183,284, which caused a change in the edited amino acid from isoleucine to threonine, and was observed at this mutation site in 29% of individuals with black wool and 71% of individuals with non-black wool. In the study of Vasu [35] et al., melanin was associated with black coat color and high-altitude ultraviolet protection in animals, and the association between *TYRP1* gene and black wool production in Qira sheep may be related to the survival of Qira sheep on the edge of the desert. In addition, the results of Zhang [36] et al. showed that *TYRP1* gene was significantly associated with sheep pigmentation compounds. This suggests that changes in wool color in Qira sheep are associated with the *TYRP1* gene.

In addition we identified the *PARD3* and *CDH3* genes, which have been added to the black gene pool, among the relevant candidate genes, and we hypothesize that these genes also affect the variation in black wool color in Qira sheep.

Par-3 series of battery polarity regulators (*PARD3*) located on chromosome 13 in sheep, with physical location NC_056066.1 (17,821,379… 18,391,044), with high expression in the skin, was found to be involved in the regulation of melanoma by the color gene pool. And Chen et al. [37] found that *PARD3* is one of the regulation of melanin biosynthesis process with strong selective characteristics by whole genome sequencing of Hainan black sheep. Ticha et al. [38] study finds *PARD3* gene linked to melanoma development. In addition, Hajkova et al. [39] also detected the *PARD3* gene in cellular mutations in uveal melanoma, there is no description about the specific use of the *PARD3* gene in melanoma, but the present study conjectures that this gene is associated with black hair production in Qira sheep.

The calcineurin 2 gene (*CDH2*), located in stain 23 in sheep at physical location NC_056076.1 (29,012,499..29254079), is associated with delayed melanocyte differentiation, hyperpigmentation in the eye, and medial dislocation of axial melan oblasts, and is a key factor in the expression of genes associated with the extracellular matrix and focal adhesion pathways as well as epithelial in human melanoma cells to mesenchymal transition regulators [40], cutaneous dermal melanoma cells may originate from adult melanocyte stem cells and display characteristic properties of pluripotent progenitor cells [41, 42]. The *CDH2* gene is preferentially expressed in melanoma cells through [43], and the switch of calmodulin from N-calmodulin (*CDH2*) to K-calmodulin (*CDH6*) is essential for neural crest cell formation, migration, and segregation of neural tubes into melanocytes3 [44]. A switch in calreticulin from N-calreticulin (*CDH2*) to K-calreticulin (*CDH6*) was found to be critical for neural crest cell formation, migration and segregation from the neural tube into melanocytes [44]. This gene has not been reported much in melanin formation and pigmentation in sheep, but it was shown to be in the melanogenic gene pool and was shown in each of the analyses in this study.

Among the significant candidate genes enriched with black wool formation in this study, we found that the gene *DACH1* [45] was associated with litter size in sheep, the genes *ARRDC4* [46] and *PRKD1* [47] were associated with reproductive traits, and *RIMS1* [48], *ZP4* [49] and other genes are associated with pregnancy and embryonic development, *RPL17* [50], *MDFIC* [51], *PCDH15* [52], *THSD7A* [53] and other genes are associated with fleshing and growth traits, *KLF12* [54] and other genes are associated with adipose differentiation and development, *CCND2* [55] gene is associated with testicular development in sheep. *DSC3* [56] and *INTU* [57] genes are associated with hair development, *MYO16* [58] gene is associated with mammary gland development, *TMEM132C* [59] and *OSBPL6* [60] genes are associated with milk production traits. The above results provide a new idea for the study of the production, growth and development of Qira sheep.

Through PCA, NJ-tree and admixture analyses, it was found that Qira sheep could not be classified according to wool color in different wool color genetics. There are fewer studies on wool color related genes in sheep. In this study, we found 71 genes related to wool color formation, which supplemented the theoretical basis of black sheep selection, and provided a reference for the study of the mechanism of black wool formation in Qira sheep, as well as for the conservation of germplasm resources in Xinjiang sheep.

Conclusions

In this study, we carried out analysis by PCA, NJ-tree and admixture analysis for three wool color populations of Qira sheep, and found that Qira sheep could not be

classified according to wool color in different wool color genetics. The genes related to wool color in sheep are less studied and in this study we found 71 genes related to wool color formation. A deeper study found that the variation of wool color in Qira sheep was associated with three missense mutations (2:81,183,168, 2:81,183,281 and 2:81,183,284) generated on the *TYRP1* gene, which supplemented the theoretical basis of black sheep selection and provided a reference to study the mechanism of black wool formation in Qira sheep, as well as for the conservation of germplasm resources in Xinjiang sheep.

Abbreviations

Fst	Fixation index
xp-EHH	Extended haplotype homozygosity
iHS	Integrated haplotype homozygosity score
Pi	Nucleotide diversity
B	Black
Y	Yellow–Brown
G	Grey

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11233-5.

Supplementary Material 1.

Acknowledgements
The authors would like to thank Xinjiang Jinken Aoqun Agricultural and Animal Husbandry Technology Co. for providing the experimental materials and the Jinken Pastoral Meat and Sheep Research Institute for providing the experimental site.

Authors' contributions
Data curation, Xuechen Zhang; Formal analysis, Wen Zhou, Xiaopeng Li, Xuechen Zhang and Xinyu Bai; Funding acquisition, Shudong Liu; Investigation, Wen Zhou; Methodology, Wen Zhou and Ruizhi Yang; Project administration, Shudong Liu; Resources, Wen Zhou and Xinyu Bai; Software, Wen Zhou and Cheng-long Zhang; Supervision, Zhipeng Han; Visualization, Wen Zhou and Xiaopeng Li; Writing – original draft, Wen Zhou; Writing – review & editing, Wen Zhou, Xuechen Zhang, Zhipeng Han and Lijun Zhu.

Funding
This study was funded by grants from the Natural Science Foundation of China (NO:32060743), Bingtuan Science and Technology Program (NO: 2022CB001-09), Science and Technology Action for Industrial Development for Rural Revitalisation in the Autonomous Region (NO:2022NC110).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The animal study underwent thorough review and received approval from the Ethics Committee of the College of Animal Science and Technology of Tarim University (protocol code: 2023039).

Competing interests
The authors declare no competing interests.

Received: 2 November 2024 Accepted: 10 January 2025
Published online: 06 February 2025

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