Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Genome-wide association study and gene ontology for growth and wool characteristics in Zandi sheep

Hossein Mohammadi[1,2], Seyed Abbas Rafat[1*], Hossein Moradi Shahrebabak[3], Jalil Shodja[1] and Mohammad Hossein Moradi[2]

[1]Department of Animal Science, Faculty of Agricultural Sciences, University of Tabriz, Tabriz, East Azerbaijan, Iran.

[2]Department of Animal Sciences, Faculty of Agriculture and Natural Resources, Arak University, Arak, Markazi, Iran.

[3]Department of Animal Sciences, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Alborz, Iran.

Abstract Genome-wide association studies (GWAS) is a major procedure for studying the genetics of complex economically important traits in sheep. The objective of this study was to determine the genomic regions affecting some growth traits and wool characteristics in Zandi sheep. This study is GWAS implementing a medium-density single nucleotide polymorphism (SNP) panel to determine the putative chromosome area affecting some growth and wool traits in a fat-tailed sheep breed, simultaneously. We used a selective genomic approach sampling DNA from animals at the extreme ends using the estimated breeding values derived from a total population size of over 5,000 animals. The examined phenotypic data included the birth weight, weaning weight, 6, 9, and 12-months after birth weight, pre- and post-weaning average daily gain, fiber diameter (micron), prickle factor (%), staple length (mm), kemp (%) and medullated fiber. Genome-wide association analyses were performed based on the mixed linear model. Twenty-three regions, in which four were associated with more than one trait, located on 12 chromosomes were associated with the studied growth and wool traits ($P<5\times10^{-6}$). These genomic regions overlapping with *KCNIP4, PPARGC1A, ASAP1, ANK2, WWOX, SYNE1, FBXO5, AKAP6, FABP3, ANGPTL4, ATP6V1B2, PARK2, and KRTAP11-1* genes, were associated with postnatal growth, regulation of metabolic pathways, skeletal muscle differentiation, and bone growth. Gene ontology term enrichment analysis revealed that genes involved in positive regulation of muscle structure, and muscle tissue development were over-represented in the identified candidate genes.

Keywords: candidate gene, genome-wide association, growth, sheep, wool

Paper type: Research Paper

***Corresponding author,**
E-mail address:
rafata@tabrizu.ac.ir

Received: **24 Apr. 2020,**
Accepted: **24 Mov. 2020,**
Published online: **19 Dec. 2020.**

© The authors, 2020.

Introduction

Until now, 3411 QTLs associated with economically important traits in sheep have been reported and deposited in the sheep QTLdb (http://www.animalgenome.org/cgi-bin/QTLdb/OA/index, Release 42, Aug. 27; 2020). These findings have significantly improved our knowledge of the genetic architecture of sheep growth and wool traits. Nevertheless, most of the QTL studies, which were based on marker-QTL linkage analysis captured a low proportion of the genetic variation for traits mostly because of the low density of the markers historically used in QTL mapping studies (Zhang et al., 2012). Accordingly, the calculated confidence intervals for most reported QTLs are too spacious to enable the identification of suitable positional candidate gene(s) for the majority of QTL (Zhang et al., 2012).

Several regions of the ovine genome associated with economic traits have been reported in sheep (Al-Mamun et al., 2015; Bolormaa et al., 2016; Ebrahimi et al., 2017; Matika et al., 2016). Also, recently several GWAS studies identified the genetic variants for productive and reproductive traits in fat-tail sheep (Abdoli et al., 2019; Gholizadeh et al., 2015; Pasandideh et al., 2018; Pasandideh et al., 2020; Xu et al., 2017). One of the reasons for the imperfect knowledge on the sheep genome may be the previous release of the *Ovis_aries_v3.1* reference genome sequence in 2012 (Zhang et al., 2013). According, the latest assembly of the sheep genome (*OAR v4.0*) was developed (Oar_v4.0 in NCBI assembly database), including a total assembled length of 2.61 Gb, consisting of 20,645 protein-coding genes (NCBI Ovis aries Annotation Release 103).

Zandi sheep is a fat-tailed sheep breed comprising a population of more than 2 million heads (Bohlouli et al., 2013). This breed is double-coated and produces carpet wool, which contains medullated fibers used in the manufacture of handmade carpets.

The aim of study was to carry out GWAS using genome-wide SNP markers in Zandi sheep to identify the candidate region(s) associated with several growth and wool traits.

Materials and methods

Animals

The animals were sampled from the Zandi Sheep Breeding Station (Khojir), located in Tehran province (1547 m above mean sea level and 35°45'E and 51°40'N). The flock was established in mid-1980s. Genetic sampling of the population began in 1992 and was used to build a genetic pedigree. By 2015, the pedigree contained 975 dams and 264 of sire links involving 7,147 sheep.

The individuals for genotyping were selected based on complete of records, and from the two tails of the phenotypic distribution for growth traits (He et al., 2017). Therefore, 96 samples with complete records over multiple body weights and wool production were selected. The 96 animals belonged to 33 half-sib families with unrelated dams at possible.

The studied traits included the birth weight (BWT; kg), weaning weight (WWT; kg), 6-month weight (6-MW; kg), 9-month weight (9-MW; kg), 12-month weight (YW; kg), preweaning average daily gain (PRWG; kg), postweaning average daily gain (POWG; kg), mean fiber diameter (MFD; µm), fiber diameter coefficient of variation (FDCV; %), prickle factor (PF; the percentage of fibers with diameters greater than 30 microns), staple length (SL; mm), percentage of kemp fiber (KEMP; %), and percentage of medullated fiber (OCF; %). Descriptive statistics of the phenotypic observations of 13 growth and wool traits are presented in Table 1.

DNA extraction, genotyping and quality control

DNA was extracted from blood samples by applying a modified salting-out protocol (Helms, 1990). The criteria of DNA quality control were DNA concentration, which must be larger than 50 ng/µL, with the ratio of OD260/OD280 in the range of 1.7–1.9. All animals were genotyped using the Illumina Ovine SNP50 BeadChip (Illumina Inc., San Diego, CA, USA, 2015), with standard procedures at GeneSeek Inc. Lincoln, NE, USA. To map the SNPs to the updated gene annotation version for sheep, we rearranged the SNPs in accord with *Ovis_aries_v4.0* (last accessed 1 June, 2016).

To guarantee the high quality of the SNP data, a series of quality control measures was carried out using the PLINK v1.70 (Purcell et al., 2007). The individuals and SNPs were further analysis as follows: individual was excluded if (1) more than 10% of the genotypes were missing, or (2) it was a duplicate sample. An SNP was removed if (1) its call rate was less than 95%, (2) its minor allele frequency (MAF) was less than 1%. For the remaining SNPs, any outliers from the Hardy–Weinberg equilibrium (P value of less than 10E-6) across all animals were used to identify the genotyping errors.

Genome-wide association analysis (GWAS)

GWAS was carried out using TASSEL 5.0 based on the mixed linear model of (1).

Table 1. Descriptive statistics of 13 sheep growth traits and wool characteristics

Traits	Mean	Standard deviation	Minimum	Maximum	Standard error
Birth weight (kg)	4.10	0.77	2.10	6.28	0.068
Weaning weight (kg)	25.69	4.80	9.00	30.00	0.484
6-month weight (kg)	38.14	5.29	13.10	59.00	0.519
9-month weight (kg)	45.90	5.98	24.04	62.00	0.569
12-month weight (kg)	58.46	6.79	30.13	69.25	0.604
Preweaning average daily gain (kg)	0.197	0.04	0.08	0.34	0.004
Postweaning average daily gain (kg)	0.126	0.07	0.03	0.41	0.007
Mean Fiber diameter (μm)	29.85	3.25	22.40	39.04	0.032
Fiber diameter coefficient of variation (%)	43.12	7.84	19.0	68.35	0.764
Prickle factor (%)	27.04	10.42	12.04	43.10	0.998
Staple length (cm)	11.25	3.92	6.00	19.00	0.035
Kemp (%)	5.81	1.16	1.89	8.96	0.015
Outer coat fiber (%)	2.37	2.10	0.97	9.33	0.020

$$y=Xb+Ws+Zu+e \quad (1)$$

where $\boldsymbol{y}$ is the vector of the phenotypic records, $\boldsymbol{b}$ is a vector containing fixed effects birth type, age of dam, birth year, $\boldsymbol{s}$ is a vector of the SNP effect, $\boldsymbol{u}$ is a vector of the polygenic effect. X, W and Z are the known design matrices for the fixed effects $\boldsymbol{b}$, $\boldsymbol{s}$ and $\boldsymbol{u}$, respectively. First column of X matrix is 1s for overall mean. Here we assumed that $\boldsymbol{u}$ and $\boldsymbol{e}$ follow a normal distribution. Logarithm transformation was used for FDCV, PF, KEMP, and OCF.

Statistical inference and population stratification assessment

The Bonferroni method was used to adjust for the multiple SNP loci. Commonly, if estimates were overinflated, we used the genomic inflation factor λ using the PLINK (Chang et al., 2015). We also appraised their deviation from the expected distribution of no SNPs being associated with the trait of interest using a quantile-quantile (Q-Q) plot (Mohammadi et al., 2018).

Gene annotation and bioinformatics analyses

The closest genes to significant SNPs within a 500-kb window on both sides of the SNP location were identified. The positions of the annotated genes were extracted from the latest sheep genome *Oar_v4.0* assembly along with the NCBI annotation release 102 of the sheep genome.

Gene-specific functional analyses were performed by GeneCards (www.genecards.org) and NCBI database consultation.

Functional annotations Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway were assigned to the genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) Bioinformatics Resources V6.8 (available from http://david.abcc.ncifcrf.gov, last accessed Oct. 2016) (Huang et al., 2009). Because of the incomplete GO annotation for sheep, human orthologous Ensembl IDs were also used to maximize recognition by the DAVID tool. Only GO terms with a significant *P-value* of 0.05 (Benjamini–Hochberg FDR corrected) were retained.

Orthologous characterization of positional candidate genes

We initially investigated for human-annotated genes that are associated with the 'body mass index' phenotype using the *GUILDify* web (*http://sbi.imim.es/web/GUILDify.php*).

Results

From the initial set of 52 446 SNPs, 3320, 1568 and 147 SNPs were removed because of MAF, the call rate, and Hardy-Weinberg equilibrium reasons, respectively. Two individuals were excluded with a call rate lower than 0.9. After quality control, 100 individuals and 47 411 SNPs were kept for the following analysis. The number and the average distances between adjacent SNPs on each chromosome, before and after filtering, are shown in Figure 1.

Q-Q plots comparing the observed distribution of –log (P-value) to the expectation under null hypothesis are shown in Figure 2: The plots show a distribution close to the expected distribution line for growth and wool traits along with the λ values< 1.1, showing the inflation acceptable, and then the genomic control correction was used to correct for the inflation.

According to the considered significance threshold

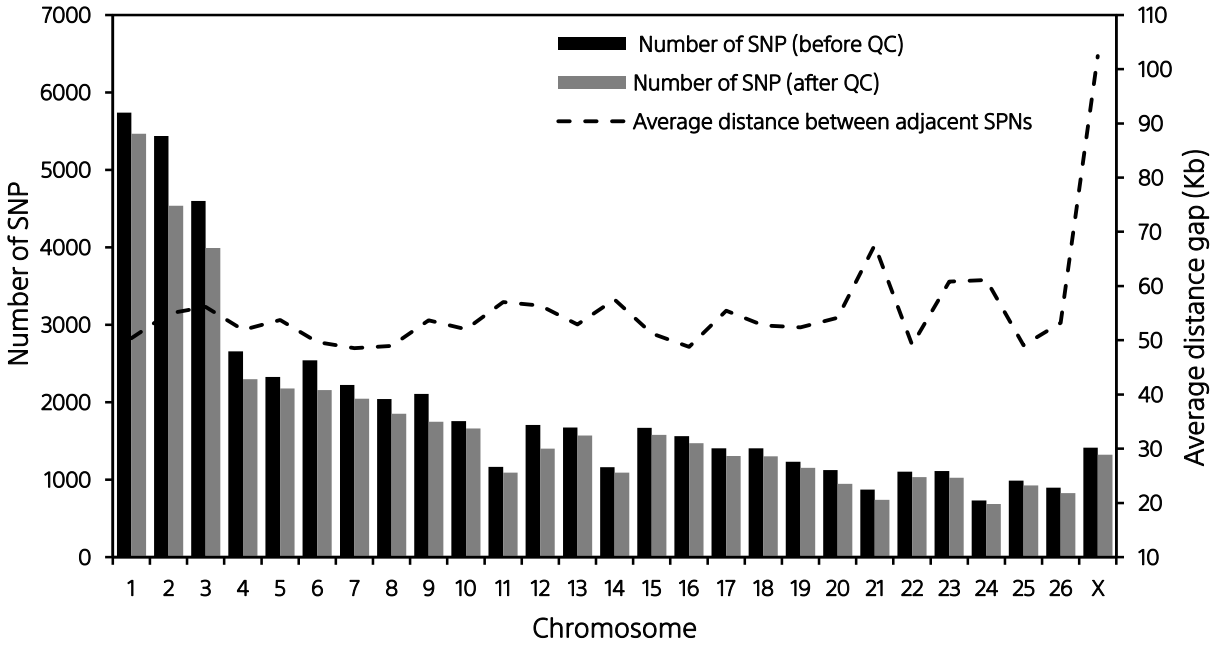

Figure 1. Number of SNPs and average distance between adjacent SNPs per chromosome after quality control process. The average marker distance in all autosomes is about 50 kb. The marker distance in the sex chromosome is about 100 kb.

($P<5\times10^{-6}$), 23 regions were associated with studied growth and wool traits (Table 2). These markers were distributed on OAR 1, 2, 3, 5, 6, 7, 8, 9, 11, 13, 14, and 18. No significant SNPs were identified for SL, KEMP and OCF. The–log (P value) for all SNP markers of each trait is shown by a Manhattan plots in Figure 3. The genes and previously reported QTLs located near the SNPs associated with growth and wool traits also presented in Table 2. The gene ontology (GO) term analyses of the positional candidate genes are shown in Table 3.

Discussion

Birth weight

A region on OAR 6 located at 40.6 Mb was associated with BWT (Table 2). A gene near this SNP is *KCNIP4 (*Potassium Voltage-Gated Channel Interacting Protein 4). *KCNIP4* is a member of the family of voltage-gated potassium channel-interacting proteins (KCNIP). Potassium channels have extensive physiological adjustment functions, including neurotransmitter release, contraction of smooth muscle, heart rate adjustment, and insulin secretion. Therefore, we hypothesize that *KCNIP4* has a significant effect on sheep growth. This region overlapped with a QTL, which has been reported to affect birth weight in Kermani sheep (Esmailizadeh et al., 2010) and body weight (slaughter) in crosses between Awassi and Merino sheep (Cavanagh et al., 2010). Pasandideh et al. (2018) also found associations of this gene with average daily gain in 3-12 months in Baluchi Sheep.

Weaning weight and pre-weaning ADG

Four genomic regions were identified associated with WWT in this study, where two of these regions were also associated with PRWG (Table 2). One of these markers was OAR6_48108133.1, located at 43.0 Mb of OAR 6. On chromosome 6, the only gene identified close to this marker was *PPARGC1A*, located 62.1 kb upstream. *PPARGC1A* transcriptionally activates complex pathway of mitochondrial biogenesis in lipid metabolism. Many studies have reported associations between PPARGC1A gene and production traits (Li et al., 2014).

The second SNP associated with WW and PRWG traits was located at 23.6 Mb of OAR 9. Genes located close to this SNP included *ASAP1* (ArfGAP with SH3 domain, Ankyrin repeat and PH domain 1), *FAM49B* (Family with Sequence Similarity 49 Member B), and *GSDMC* (Gasdermin C). The most important gene identified in this region was *ASAP1*. This gene belongs to the ArfGAP with SH3 Domain, Ankyrin Repeat and PH Domain (ASAP) complex family. The *ASAP1* gene is a candidate gene in sheep since it is located on the OAR9, where

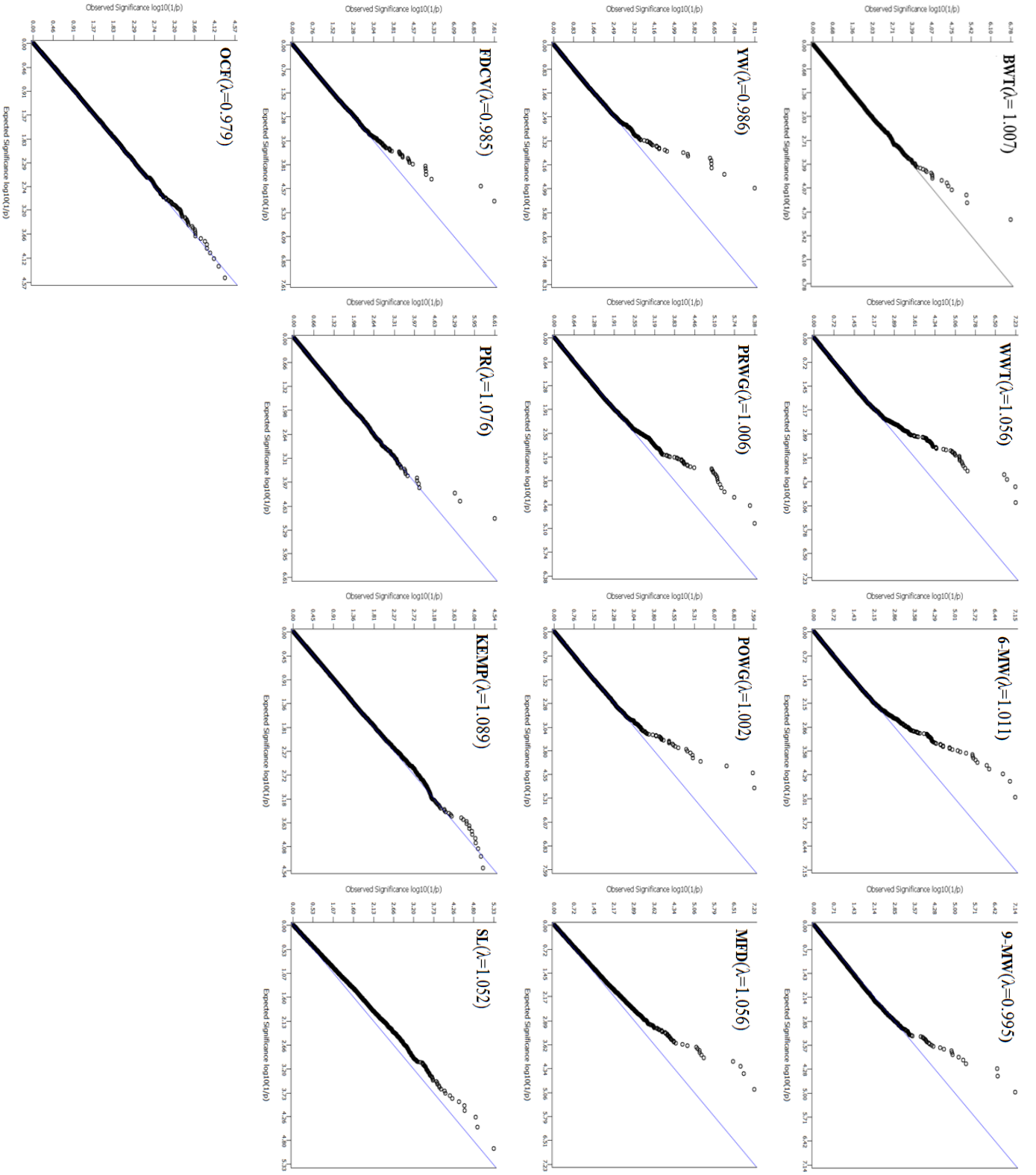

Figure 2. Quantile–quantile (Q-Q) plots for the genome wide association study of growth and wool traits. The straight line in the Q-Q plots indicates the distribution of SNP markers under the null hypothesis, and the skew at the edge indicates that these markers are more strongly associated with the traits than would be expected by chance. BWT=birth weight; WWT=weaning weight; PRWG = pre-weaning ADG; 6-MW= 6-month weight; 9-MW = 9-month weight; YW=12-month weight; POWG=post-weaning ADG; MFD=mean fiber diameter; FDCV=fiber diameter coefficient of variation; PR=Prickle Factor; SL=staple length; KEMP=percentage of kemp fibre; OCF= percentage of medullated outer coat fiber.

Table 2. Genes and previously reported QTLs located near the significant SNPs associated with growth and wool traits in sheep

Trait(s) _Chr_Mb_ SNP ID[a]	Genes in ±500 kb[b]	Distance, kb[c]	QTL[d]	PubMed ID
BWT _6_40.6_ OAR6_45381991.1	*SLIT2, PACRGL,* KCNIP4	+534.30	HCW and BW(Slaughter)	20846385
WWT,PRWG_6_43.0_ OAR6_48108133.1	PPARGC1A	-62.10	HCW BW(Slaughter)	20846385
WWT,PRWG_9_23.6_ s09785.1	ASAP1, *FAM49B, GSDMC*	+355.71	HCW and LMA	20846385
WWT_2_90.6_OAR2_97242630.1	*DMRTA1*	-	HCW	20846385
WWT_2_92.2_OAR2_99110156.1	*IZUMO3*		MFP	19397522
6-MW_2_43.2_OAR2_45999847.1 6-MW_2_43.1_ s70232.1 6-MW_2_43.1_ s75980.1	*CCAR2, SORBS3, PPP3CC, PIWIL2, PHYHIP*	-	-	-
6-MW_6_12.5_OAR6_15203898.1	ANK2, *CAMK2D, SCARNA17, SCARNA18, U6*	Within	IFM, CFP, LMYP	20846385
6-MW_14_5.1_ s70110.1	WWOX	+535.13	BW and DP	20846385
9- MW_8_76.0_OAR8_82107007.1	*ESR1, SYNE1, MYCT1, VIP,* FBXO5, *MTRF1L, RGS17, RGS17*	Within -344.68	IFA	20846385
9-MW_18_41.4_OAR18_44175536.1	*NUBPL, ARHGAP5,* AKAP6, *U6*	-81.68	CFP	20846385
9-MW_14_9.2_OAR14_9604741.1	-	-	DP	20846385
YW, POWG_2_235.5_s00417.1 YW_2_235.5_DU382510_248.1 YW_2_235.5_ s47808.1	*SERINC2,* FABP3, *SNRNP40, NKAIN1, PUM1, SDC3, LAPTM5, MATN1*	+388.32	HCW and BW(Slaughter) BD	20846385 14555257
YW_2_68.4_ OAR2_73090654.1	*DOCK8, KANK1, DMRT1, DMRT2, DMRT3*	-	HCW and BW(Slaughter)	20846385
YW_5_14.1_ s67215.1	*INSR, PEX11G, PEX11G, C19orf45, ZNF358, MCOLN1, PNPLA6, CAMSAP3, XAB2, PET100, PCP2, STXBP2, RETN, MCEMP1, TRAPPC5, FCER2, CLEC4G, EVI5L, LRRC8E, MAP2K7, TIMM44, FBN3, CERS4, KANK3, CD320, RPS28,* ANGPTL4	+496.33	BW (birth)	ISU0054
YW_7_81.0_ s67114.1	*DPF3, RGS6*	-	MFP	23810588
POWG_2_45.0_ OAR2_47716594.1	*LZTS1,* ATP6V1B2, *SLC18A1, U6*	-310.04	MFP	19397522
POWG_8_84.2_OAR8_90924913_X.1	PARK2	+445.36	BW(birth)	17542849
MFD_1_116.2_OAR1_125367772.1 MFD_1_122.4_s32542.1	*TMCO1, UCK2, FAM78B, SCAF4, TIAM1,* KRTAP11-1	+86.73	-	-
MFD_13_8.7_OAR13_9727532.1 MFD_13_82.6_ OAR13_88824402.1	*CBLN4*	-	-	-
FDCV, PR_11_38.5_ s58110.1	*TTLL6, HOXBs, HOXB-AS1, HOXB-AS2, SNX11, NFE2L1, SCRN2*	-	FDCV	22444653
FDCV _3_218.6_s18720	*EFCAB6, SULT4A1, PNPLA3, PNPLA5, SAMM50, PARVB, PARVG, KIAA1644, PRR5*	-	-	-

[a]BWT=birth weight; WWT=weaning weight; PRWG = preweaning average daily gain; 6-MW= 6-month weight; 9-MW = 9-month weight; YW=12-month weight; POWG=postweaning average daily gain; MFD=mean fiber diameter; FDCV=fiber diameter coefficient of variation; PR=Prickle Factor.
[b]The most important gene(s) related to traits is labeled bold.
[c]Positive value denotes the gene located downstream of SNP, negative value denotes the gene located upstream of SNP. Sign "_" indicated none of them demonstrated any direct relationship with growth and wool traits.
[d]HCW=hot carcass weight; BW =body weight; LMA= Longissimus muscle area; MFP=milk fat percentage; IFM= internal fat amount; CFP=carcass fat percentage; LMYP= Lean meat yield percentage; DP=dressing percentage; IFA= internal fat amount; BD=bone density.

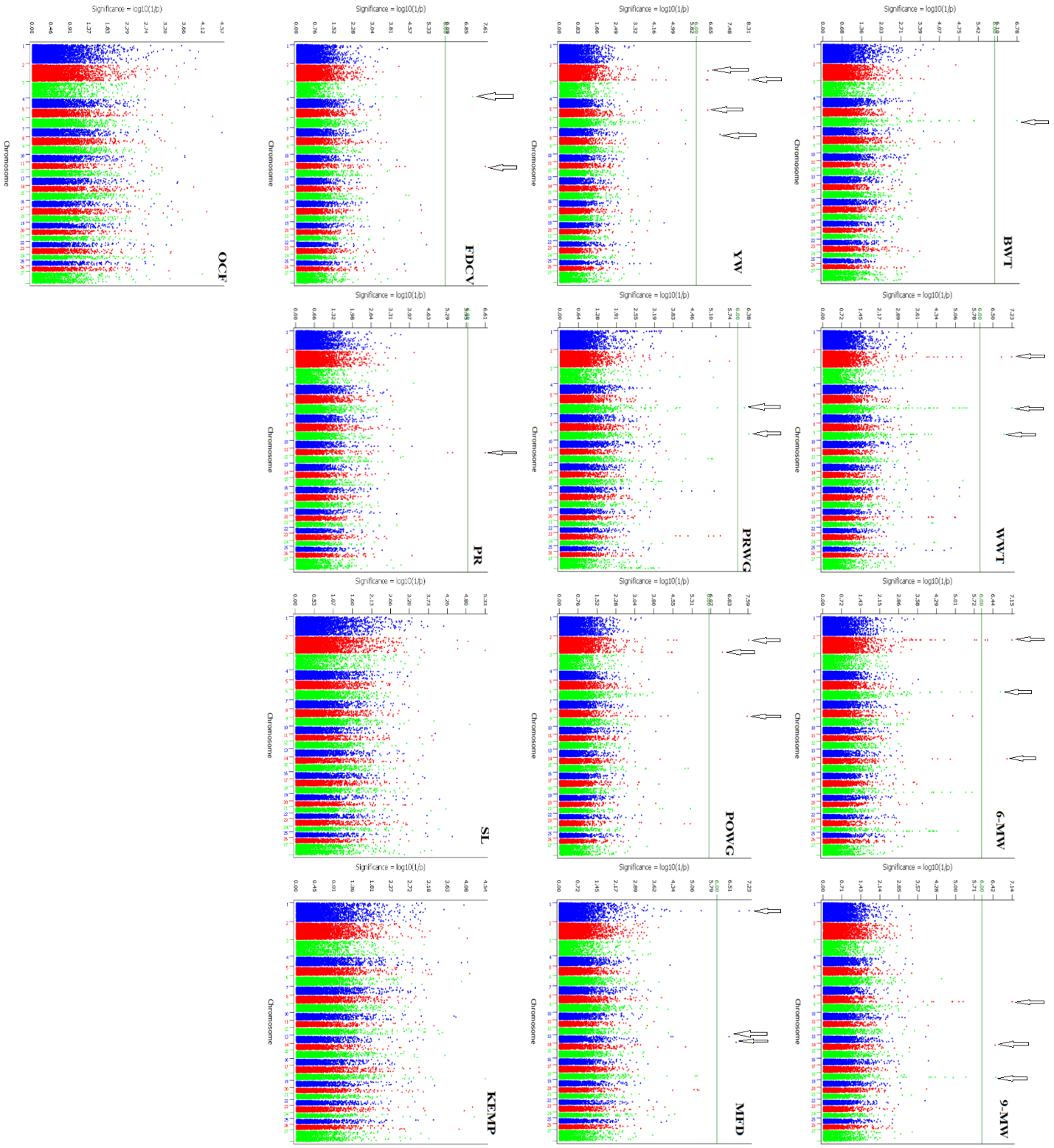

Figure 3. Manhattan plots of the log10 (1/P-values) (y-axis) for the genome wide association study of growth and wool traits .The genomic position with chromosome number is represented along the x-axis. The horizontal line indicates the significant threshold for significant associations ($P<5\times10^{-6}$). BWT=birth weight; WWT=weaning weight; PRWG = pre-weaning ADG; 6-MW= 6-month weight; 9-MW = 9-month weight; YW=12-month weight; POWG=post-weaning ADG; MFD=mean fiber diameter; FDCV=fiber diameter coefficient of variation; PR=Prickle Factor; SL=staple length; KEMP=percentage of kemp fibre; OCF= percentage of medullated outer coat fiber.

Table 3. List of significant gene ontology (GO) terms from GO analysis of positional candidate genes

GO[a]	Term ID	Term name	Gene list	p-value
GOTERM_BP_FAT	GO:1901863	positive regulation of muscle tissue development	PPARGC1A, AKAP6, PARK2	4.0E-2
GOTERM_BP_FAT	GO:0061061	muscle structure development	AKAP6, ANK2, SYNE1	4.7E-2
GOTERM_BP_FAT	GO:0019217	regulation of fatty acid metabolic process	PPARGC1A, FABP3	5.0E-2
GOTERM_BP_FAT	GO:0046889	positive regulation of lipid biosynthetic process	PPARGC1A, FABP3	3.6E-2
GOTERM_BP_FAT	GO:1904427	positive regulation of calcium ion transmembrane transport	AKAP6, ANK2	3.5E-2
GOTERM_BP_FAT	GO:0009893	positive regulation of metabolic process	AKAP6, PPARGC1A, WWOX, ANK2, FABP2, PARK2	2.3E-2
GOTERM_BP_FAT	GO:0034765	regulation of ion transmembrane transport	AKAP6, PPARGC1A, ANK2, KCNIP4	1.4E-3
ke	KEGG:03320	PPAR signaling pathway	ANGPTL4, FABP3	5.00E-2

[a]BP: Biological process, ke: KEGG pathway

there are reports of QTLs for meat production traits including, hot carcass weight and Longissimus muscle area (Cavanagh et al., 2010).

6-month weight (6-MW)

Five SNP markers located in three genomic regions were associated with 6-MW. Three of these markers, including OAR2_45999847.1, s70232.1, and s75980.1, were located at 43.1-43.2 Mb of OAR 2. The genes *CCAR2* (Cell Cycle and Apoptosis Regulator 2), *SORBS3* (Sorbin and SH3 Domain Containing 3), *PPP3CC* (Protein Phosphatase 3 Catalytic Subunit Gamma), *PIWIL2* (Piwi Like RNA-Mediated Gene Silencing 2), and *PHYHIP* (Phytanoyl-CoA 2-Hydroxylase Interacting Protein) were located in this region of OAR 2.

At 12.5 Mb of OAR 6, the OAR6_15203898.1 SNP has located within the *ANK2* (Ankyrin 2) gene. *ANK2* is a member of the ankyrin family. The gene *ANK2* is located on the OAR6, where there are reports of QTLs for meat quality traits, including internal fat content, carcass fat percentage, and lean meat yield percentage (Cavanagh et al., 2010).

The last marker associated with 6-MW on OAR 14, was located near the *WWOX* (WW domain-containing oxidoreductase) gene. *WWOX* are promising candidate genes for bone mineral content, muscle metabolism and, backfat thickness. Therefore, it can be assumed that *WWOX* variants could also affect the growth traits in sheep. A QTL affecting the bone weight in the carcass and dressing percentage has previously been reported in the same position in sheep (Cavanagh et al., 2010).

9-month weight (9-MW)

Three genomic regions were associated with 9-MW (Table 2). At 76.0 Mb of OAR 8, the OAR8_82107007.1 SNP was located within the *SYNE-1* (Spectrin Repeat Containing Nuclear Envelope Protein 1) gene. Other genes located in this region were *ESR1* (Estrogen Receptor 1), *MYCT1* (Myc Target 1), *VIP* (Vasoactive Intestinal Peptide) *FBXO5* (F-Box Protein 5), *MTRF1L* (Mitochondrial Translational Release Factor 1 Like) and *RGS17* (Regulator of G-Protein Signaling 17). The most important genes identified in this region were *SNYE-1* and *FBXO5*. *SYNE-1*, which are associated with cardiac and smooth muscle cells. A recent study by Gholizadeh et al. (2015) reported a possible influence of the *SNYE1* gene on the yearling body weight in Baluchi sheep.

The region identified on OAR 18 in this study was located near the *NUBPL* (Nucleotide Binding Protein Like), *ARHGAP5* (Rho GTPase Activating Protein 5), *AKAP6* (A-Kinase Anchoring Protein 6), and *U6* (U6 spliceosomal RNA) genes. Among these genes, *AKAP6* presented exciting features. One family of well-studied scaffolding proteins is composed of the A-kinase Anchoring Proteins (AKAPs). One of AKAPs, *AKAP6*, also known as muscle AKAP (*mAKAP*), is a regulatory factor for skeletal muscle differentiation. Previously reported QTLs found in these genomic regions were associated with carcass fat percentage (Cavanagh et al., 2010).

Yearling weight and post-weaning ADG

Four regions associated with yearling weight were identified, where one of these regions was also associated with POWG (Table 2). Three SNP markers, namely, s00417.1 (associated with both characteristics), DU382510_248.1, and s47808.1 on OAR 2 were located near *SERINC2* (Serine Incorporator 2), *FABP3* (Fatty Acid Binding Protein 3), *SNRNP40* (Small Nuclear Ribonucle-

oprotein U5 Subunit 40), *NKAIN1* (Sodium/Potassium Transporting ATPase Interacting 1), *PUM1* (Pumilio RNA Binding Family Member 1), *SDC3* (Syndecan 3) and *LAPTM5* (Lysosomal Protein Transmembrane 5) genes. The most important gene identified in this region was *FABP3*.

The other SNP marker associated with YW was found in a gene-dense region of OAR 5. The analysis of this region revealed the presence of 27 genes (Table 2). Among these 27 genes, *ANGPTL4* presented exciting features.

Three genomic regions were associated with POWG in this study, of which, one located on OAR2 was familiar with YW; however, as shown in Table 2, other SNP markers were located at 45.0 and 84.2 Mb of OAR 2 and 8, respectively. The closest gene to the genomic region on OAR2 was *ATP6V1B2* (ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2). *ATP6V1B2* is involved in transmembrane transport and generation of precursor metabolites and energy.

The only gene identified close to the genomic region on OAR8 was *PARK2* (Parkin RBR E3 Ubiquitin Protein Ligase), located 445.3 kb downstream. This gene catalyzes protein ubiquitylation consequence in the targeting of proteins toward different cellular fates, with proteasome-mediated proteolytic degradation.

Fiber diameter (FD)

Four genomic regions associated with FD were identified on OAR 1 and OAR13 in this study. The most important gene identified close to the SNP markers located on OAR1 in these regions was *KAP11-1*. This gene belongs to the family of keratin-associated proteins (KAPs). So far, more than 100 *KAP* genes, grouped into 30 KAP different families, have been identified in mammalian species (Rogers et al., 2002).

The other SNPs associated with FD were OAR13_9727532.1 and OAR13_88824402.1, located at 8.7 and 82.6 Mb of OAR 13, respectively. The only gene identified close to the OAR13_88824402.1 SNP was *CBLN4* (Cerebellin 4 Precursor), located 1.4 kb upstream. This gene encodes a member of a family of small secreted proteins that are involved in the regulation of neurexin signaling. Wang et al. (2014) identified two QTL on OAR13 at 26.4 Mb, controlling the mean fiber diameter trait in Chinese Merino sheep that are close to the region of our interest on OAR13.

Fiber diameter coefficient of variation and prickle factor

As shown in Table 2, two genomic regions were identified on OAR3 and OAR11 related to FDCV, where one of these regions on OAR3 may have a pleiotropic effect on the PF trait. The closest genes to the genomic region on OAR11 were *TTLL6* (Tubulin Tyrosine Ligase like 1), *HOXBs* (Homeobox B, 1-7), *HOXB-AS1* (HOXB Cluster Antisense RNA 1), *HOXB-AS2* (HOXB Cluster Antisense RNA 2), *SNX11* (Sorting Nexin 11), *NFE2L1* (Nuclear Factor, Erythroid 2 like 1), and SCRN2 (Secernin 2). Although direct functional effects have not been reported for these genes with fiber characteristics, the region of our interest is close to a region containing keratin gene family associated with cytoskeletal components of epithelial cells (1.08 Mbp upstream) namely *KRT24*, *KRT25*, *KRT26*, *KRT27, KRT28*, and *KRT10*. As mentioned previously, keratins play an essential role in the structure of the hair shaft. The other SNP markers that were exclusively associated with FDCV and PR, were located at 218.6 Mb of OAR 3. The genes close to this genomic region are shown in Table 2, and no individual candidate genes related to fiber characteristics were identified.

Staple length, KEMP, and OCF

No genomic region was detected for SL, KEMP, and OCF traits. This may be due to the relatively small sample size and the rigorous significant threshold used in this study. Furthermore, the results may suggest a different genetic architecture for these traits that are controlled by several genes, each with a small effect, so that only 7 QTLs have so far been reported for SL and none QTL for KEMP, and OCF (Ponz et al., 2001; Esfandyari et al., 2011).

Identification of the regions with pleiotropic effect

The regions were found to be important in more than one trait, including WWT and PRWG although the significance levels varied with different traits. Polymorphisms that affect complex traits may influence multiple traits. The results of this study are in consistent with some previous studies on sheep, human, cattle, and chicken (Al-Mamun et al., 2015; Bolormaa et al., 2016).

Recently, Bolormaa et al. (2016), by combining 56 single GWAS study in sheep for carcass composition in a meta-analysis, detected a region on OAR6 at 37.5 Mb with pleiotropic effects on carcass, skeletal weights, lean meat yield, decreased dressing percentage and fatness. Weng et al. (2016) detected one SNP on BTA6 at 38.6 Mb, explaining 6.9% of the genetic variance on birth weight and 1.16% on weaning weight in Brangus beef cattle. This result is in agreement with the 42.6–43.6 Mb region on OAR6 that we found to be significantly associated with WWT and PRWG.

Candidate genes within these pleiotropic regions, along with the genes most important at different ages (Table 2), were interrogated for GO category and KEGG. Gene ontology showed significant enrichment for functional categories related to tissue development (Table 3).

In general, the results of this study revealed some novel genomic regions associated with growth (e.g., genomic regions located on OAR 2, 5, 6, and 18) and wool quality (OAR1) that had not been previously identified. They can be the subject of further study on genetic architecture of these economically important traits in sheep production.

Conclusion

This study implemented a medium-density SNP panel to identify the putative genomic regions affecting the growth traits and wool characteristics in a fat-tailed sheep breed. A total of 23 distinct regions were associated with ten growth and wool traits at the genome-wide level. GO analysis revealed that genes included muscle structure and in muscle tissue development were overrepresented in the recognized candidate genes. Also, GO analysis showed that the identified candidate genes harbor genes that included in the PPAR signaling pathway. Overall, our findings could make the basis of follow-up studies, revealing the causal mutations underlying several growth and wool traits in fat-tailed sheep.

Acknowledgements

The authors gratefully acknowledge the Animal Breeding Center of Iran (ABCI) for access to the records and animals. The authors would also like to thank the staff of the Zandi Sheep Breeding Station for cooperation in blood and wool sampling.

Funding

Funding for OvineSNP50 BeadChip genotyping was provided by a grant from the Animal Breeding Center of Iran (ABCI), Grant no. IR 102–5513. Also, this project is supported by a research grant of the University of Tabriz (number S/856-6/3/97).

References

Abdoli, R., Mirhoseini, S., Hossein-Zadeh, N.G., Zamani, P., Moradi, M.H., Ferdosi, M.H., Gondro, C., 2019. Genome-wide association study of first lambing age and lambing interval in sheep. *Small Ruminant Research* 178, 43-45.

Al-Mamun, H.A., Kwan, P., Clark, S.A., Ferdosi, M.H., Tellam, R., Gondro, C., 2015. Genome-wide association study of body weight in Australian Merino sheep reveals an orthologous region on OAR6 to human and bovine genomic regions affecting height and weight. *Genetics Selection Evolution* 47, 66.

Bohlouli, M., Mohammadi, H., Alijani, S., 2013. Genetic evaluation and genetic trend of growth traits of Zandi sheep in semi-arid Iran using random regression models. *Small Ruminant Research* 114, 195–201.

Bolormaa, S., Hayes, B.J., van der Werf, J.H.J., Pethick, D., Goddard, M.E., Daetwyler, H.D., 2016. Detailed phenotyping identifies genes with pleiotropic effects on body composition. *BMC Genomics* 17, 224.

Cavanagh, C.R., Jonas, E., Hobbs, M., Thomson, P.C., Tammen, I., Raadsma, H.W., 2010. Mapping Quantitative Trait Loci (QTL) in sheep. III. QTL for carcass composition traits derived from CT scans and aligned with a meta-assembly for sheep and cattle carcass QTL. *Genetics Selection Evolution* 42, 36.

Chang, C.C., Chow, C.C., Tellier, L.C.A.M., Vattikuti, S., Purcell, S.M., Lee, J.J., 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* 4, 7.

Ebrahimi, F., Gholizadeh, M., Rahimi-Mianji, G., Farhadi, A., 2017. Detection of QTL for greasy fleece weight in sheep using a 50 K single nucleotide polymorphism chip. *Tropical Animal Health and Production* 8, 1657-1662.

Esfandyari, H., Aslaminejad, A.A., Rafat, S.A., 2017. Wool characteristics in the third generation of Arkharmerino × Ghezel and Arkharmerino × Moghani crossbreed sheep. *Tropical Animal Health and Production* 7, 1337-1343.

Esmailizadeh, A.K., 2010. A partial genome scan to identify quantitative trait loci affecting birth weight in Kermani sheep. *Small Ruminant Research* 94, 73–78.

Gholizadeh, M., Rahimi-Mianji, G., Nejati-Javaremi, A., 2015. Genomewide association study of body weight traits in Baluchi sheep. *Journal of Genetics* 94, 143–146.

He, L.C., Li, P.H., Ma, X., Sui S.P., Gao ,S., Kim S.W. Gu Y.Q., Huang Y., Ding N.S., Huang R.H., 2017. Identification of new single nucleotide polymorphisms affecting total number born and candidate genes related to ovulation rate in Chinese Erhualian pigs. *Animal Genetics* 48, 48–54.

Hu, Z.L., Park, C.A., Reecy, J.M., 2016. Developmental progress and current status of the Animal QTLdb. *Nucleic Acids Research* 44, 827–833.

Huang, D.W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols* 4, 44–57.

Matika, O., Riggio, V., Anselme-Moizan M., Law A.S., Pong-Wong R., Archibald A.L., Bishop S.C., 2016. Genome-wide association reveals QTL for growth, bone and in vivo carcass traits as assessed by computed tomography in Scottish Blackface lambs. *Genetics Selection Evolution* 48, 11.

Mohammadi, H., Rafat, S.A., Moradi Shahrebabak, H., Shodja, J., Moradi, M.H., 2018. An assessment of population stratification and haplotype based Genome-wide association for wool quality traits in Zandi sheep breed. *Journal of Animal Science Research* 28, 193-204. (In persian).

Pasandideh, M, Rahimi-Mianji G, Gholizadeh, M., 2018. A genome scan for quantitative trait loci affecting average daily gain and Kleiber ratio in Baluchi Sheep. *Journal of Genetics* 97, 493-503.

Pasandideh, M., Gholizadeh, M., Rahimi-Mianji, G., 2020. Identification of two novel SNPs affecting lambing traits in sheep by using a 50K SNP-Chip. *Small Ruminant Research* 191, 106193.

Ponz, R., Moreno, C., Allain, D., Elsen, J.M., Lantier, F., Lantier, I., Brunel, J.C., Pérez-Enciso, M., 2001. Assessment of genetic variation explained by markers for wool traits in sheep via a segment mapping approach. *Mammalian Genome* 12, 569–572.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Julian, M., 2007. PLINK: a toolset for whole-genome association and population-based linkage analysis. *The American Journal of Human Genetics* 81, 559-575.

Rogers, M.A., Langbein, L., Winter, H., Ehmann, C., Praetzel, S., Schweizer, J., 2002. Characterization of a first domain of human high glycine-tyrosine and high sulfur keratin-associated protein (KAP) genes on chromosome 21q22. *The Journal of Biological Chemistry* 277, 48993–49002.

Wang, Z., Zhang, H., Yang, H., Wang, S., Rong, E., Pei, W., Li, H., Wang, N., 2014. Genome-wide association study for wool production traits in a Chinese Merino sheep population. *PLoS One* 9, e107101.

Weng, Z., Su, H., Saatchi, M., Lee, J., Thomas, M.G., Dunkelberger, J.R., Garrick, D.J., 2016. Genome-wide association study of growth and body composition traits in Brangus beef cattle. *Livestock Science* 183, 4–11.

Xu, S.S., Ren, X., Yang, GL., Xie, X.L., Zhao, Y.X., Zhang, M., Shen, Z.Q., Ren, Y.L., Gao, L., Shen, M., Kantanen, J., Li, M.H., 2017. Genome-wide association analysis identifies the genetic basis of fat deposition in the tails of sheep (Ovis aries). *Animal Genetics* 48, 560-569.

Zhang, H., Wang, Z., Wang, S., Li, H., 2012. Progress of genome wide association study in domestic animals. *Journal of Animal Science and Biotechnology* 3, 26.

Zhang, L., Liu, J., Zhao, F., Ren, H., Xu, L., Lu, J., Zhang, S., Zhang, X., Wei, C., Lu, G., 2013. Genome-wide association studies for growth and meat production traits in sheep. *PLoS One* 8, e66569.