RESEARCH NOTE

Genomewide association study of body weight traits in Baluchi sheep

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Introduction

The body weight is an economically important trait in sheep. We performed a genomewide association study using Ovine 50 K SNP chip to identify the genes and chromosome regions associated with body weight in Baluchi sheep. A total of 96 blood samples from two herds along with data on weight at birth (BW), weaning (WW), six month (SMW) and yearling (YW) were collected. Markers were tested for association based on linear regression using the PLINK software. Thirteen different SNP markers reached 5% Bonferroni chromosome-wide significance levels. In this study we detected one SNP with genomewide significance effect on yearling weight on chromosome 8. All significant SNPs at chromosome-wide significance level were within or close to known ovine genes. The SNP at genomewide significance level was within gene *SYNE1*. Thus, we suggest more investigation to prove these genes as candidate genes for body weight traits in sheep.

Growth traits are economically important traits for sheep. Mapping of quantitative trait loci (QTL) is an appropriate approach and provides useful information for marker assisted selection (MAS) and gene-based selection in sheep breeding strategies. Relatively few number of QTLs have been reported in sheep. The current release (February 2014) of the Sheep QTLdb (http://www.animalgenome.org/cgi-bin/QTLdb) contains 129 QTLs for growth traits reported from a genomic study based on marker-QTL linkage analysis. QTL mapping using linkage map QTLs to large confidence intervals on the genome. As a result, use of QTL in MAS is complicated. It would be possible to exploit linkage disequilibrium (LD) to map QTL if dense markers were available. With the advent of next-generation sequencing technology and availability of tens of thousands of single-nucleotide polymorphisms (SNP markers) it is now possible to run genomewide association studies (GWAS) to identify chromosomal regions or mutations associated with traits of interest. The first report of GWAS in sheep was made by Johnston *et al.* (2011) who conducted a study using SNPs and reported the main candidate for horns as RXFP2. They described a new model of horn-type inheritance in Soay sheep, and from their results, sheep with the same horn phenotype but different underlying genotypes can be identified. The latest GWAS in sheep was reported by Zhang *et al.* (2013) who used the Illumina ovine SNP50 BeadChip and focused on the growth and meat production traits in sheep. Five genes have been reported to be the most crucial candidate genes associated with postweaning gain: *MEF2B*, *RFXANK*, *CAMKMT*, *TRHDE* and *RIPK2*.

The objective of this study was to perform a GWAS for growth traits in Baluchi sheep using the ovine SNP50 BeadChip (Illumina, San Diego, USA).

Materials and methods

Animal resources and DNA extraction

Blood samples along with data on body weights were collected from 96 Baluchi sheep (Abbasabad Sheep Breeding Station, Mashhad, Iran). Body weight was recorded at different time point including BW, WW, SMW and YW. Genomic DNA was extracted from whole blood by applying a modified salting out protocol following Miller *et al*. (1988) and DNA samples were diluted to 50 ng/μL for genotyping.

SNP genotyping and data quality control

Animals were genotyped on the Illumina ovine SNP 50K BeadChip assay using standard procedures (http://www.

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illumina.com) at the Sci-Life Lab in Uppsala, Sweden (http://www.genotyping.se). SNPs that passed the quality control criteria (genotyping frequency >95%, minor allele frequency >0.05 and Hardy–Weinberg equilibrium $P > 0.001$, calculated using PLINK ver. 1.06; Purcell *et al.* 2007) were included for further analysis.

Statistical analysis

Using PLINK an identical-by-state (IBS) correlation matrix for individuals was calculated and then dimensions were constructed using multidimensional (MDS) analysis. The eigenvalues for the first dimension (C1) of the MDS analysis along with herd effect, sex, type of birth were used as covariates in our GWAS:

$$y = SNPs + C1 + Herd + sex + type\ of\ birth + e,$$

where y is the vector of the phenotypic values of interest, SNPs the SNP genotype, C1 the eigenvalues of the first dimension of the MDS analysis and e is the residual error. Genomewide association analyses were carried out in PLINK. Linear regression analyses for body weights were performed with the first MDS component herd effect, sex, type of birth as covariates.

Significance test

A Bonferroni correction was used to control the family-wise error rate (FWER). The chromosome-wide threshold was adjusted for the number of SNP tested for each Ovis aries (OAR). The genomewide threshold was adjusted for the total number of SNPs included on all OAR. With nearly 50000 SNPs in this study, the 5% genomewide threshold was equivalent to nominal P value of 1.178×10^{-6}. The 5% chromosome-wide significance thresholds varied from the point-wise P value of 1.078×10^{-5} on chromosome 1 to 6.96×10^{-5} on chromosome 21.

Study of genes and QTLs in the candidate regions

The latest sheep genome Ovis_aries_ver. 3.1 (http://www.livestockgenomics.csiro.au/sheep/oar3.1.php permanent), and National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), were used to explore relationships between significant SNPs and ovine genes. QTL database http://www.animalgenome.org/QTLdb/cattle.html, available online was used to identify any associations of the candidate regions with published QTL in sheep.

Results and discussion

Phenotype statistics and quality control

Descriptive statistics of the phenotypic observations of the growth traits are presented in table 1. We removed 1715 SNPs with call rates less than 95% and 6933 SNPs with minor allele frequency (MAF) less than 0.05. After this cleanup of the data 42,416 SNPs passed the filtering criteria and were included in the ultimate analyses.

Genomewide association analysis

A total of 14 SNP effects involving 13 different SNP markers reached 5% Bonferroni chromosome-wide significance under the LD conditions. Among these significant SNPs, one marker associated with YW achieved genomewide significance levels (indicated in bold, table 2) and was located within known ovine gene *SYNE1* on OAR8. Thirteen SNP effects with 5% chromosome-wide significance were found on OAR5, OAR6, OAR7, OAR8, OAR13, OAR15, OAR16 and OAR25.

Birth weight (BW)

Two SNPs located 55,112–51913 bp apart from the nearest known ovine genes (*STRBP* and *TRAMIL1*) had significant effect on BW. OAR16_57881990 was located within QTL which has been reported to affect body weight (53 and 83 weeks) in Merino sheep (Raadsma *et al.* 2009). Moreover, OAR6_10161100 was located within QTL affecting lean meat yield percentage in sheep (Cavanagh *et al.* 2010). Previously other studies using microsatellite markers found QTL for body weight at birth on OAR2 (Hernández-Sánchez *et al.* 2010), OAR3 (Roldan *et al.* 2010) OAR5 (Margawati *et al.* 2006), OAR8 (Beraldi *et al.* 2007; Hernández-Sánchez *et al.* 2010) and OAR14 (Hadjipavlou and Bishop 2008).

Weaning weight (WW)

WW was nominally correlated with YW. As seen in table 2, one SNP was significantly associated with both WW and YW. Among those affecting WW, OAR7_75079311 was located within the known ovine *DAAM1* gene, and OAR15_69141695 was located 17927 bp downstream of the known ovine gene *APIP*. *DAAM1* (disheveled-associated activator of morphogenesis 1) regulates the actin cytoskeletal reorganization and plays an essential role in convergent extension movement of early vertebrate embryogenesis and later development (Sato *et al.* 2006). Zhang *et al.* (2013) reported that two SNPs on OAR3 (located within the known ovine *PFKFB4* gene) and OAR19 (located 1200 bp upstream of the known ovine gene *PLA2G6*) were associated with WW.

Six month weight (SMW)

Our GWAS identified five SNPs associated with SMW; three were located within the known ovine genes (*PHF15*, *PRSS12* and *MAN1A1*). OAR16_57881990 and OAR6_9268682 located with QTL reported to affect body weight in (Awassi × Merino) × Merino and in sheep (Cavanagh *et al.* 2010). S25202 was located within QTL which has been reported to affect BW in Indonesian thin tail × Merino

Table 1. Basic statistical information about the examined traits in Baluchi sheep.

	BW	WW	SMW	YW
Mean (kg)	4.31	23.1	32.33	39.34
SD (kg)	0.639	3.66	4.586	5.15
Minimum (kg)	2.6	15	25	28
Maximum (kg)	5.5	32	47	54
Coefficient of variation (%)	14.86	15.79	14.18	13.11

SD, standard deviation.

Table 2. SNPs with significant association with body weight.

Trait	Chromosome (CHR)	SNP	Position	The nearest known ovine genes	Distance*	*P* value
BW	16	OAR16_57881990	53180588	*STRBP* (101119162)	+55112	1.365e–005
	6	OAR6_10161100	7917701	*TRAM1L1* (101112059)	−519312	1.739e–005
WW	7	OAR7_75079311	68427400	*DAAM1* (101123636)	Within	1.739e–005
	15	OAR15_69141695	63587785	*APIP* (101105523)	−17927	3.635e–005
SMW	16	OAR16_46544413	42811161	*CDH6* (101105523)	+667130	1.044e–005
	5	s25202	43466435	*PHF15* (101103318)	Within	1.382e–005
	6	OAR6_9268682	6994356	*PRSS12* (101115965)	Within	1.719e–005
	8	OAR8_23317089	20898332	*GPRC6A* (101104322)	+22657	1.891e–005
	8	OAR8_21156854	18815533	*MAN1A1* (101103326)	Within	2.454e–005
YW	8	**OAR8_82161543**	76115771	*SYNE1* (101106269)	Within	**9.094e–008**
	13	OAR13_8646812_X	7668361	*MACROD2* (101105841)	+470339	2.29e–006
	25	s12325	40690849	*WAPAL* (101105841)	Within	1.12e–005
	13	OAR13_6751176	5949960	*ISCA1* (101105841)	−136818	1.388e–005
	7	OAR7_75079311	68427400	*DAAM1* (101105841)	Within	2.157e–005

*Positive value denotes the gene located downstream of SNP, negative value denotes the gene located upstream of SNP.
Marker associated with YW achieved genomewide significance level is indicated in bold.

× Merino backcross progeny (Margawati *et al.* 2006). PHD finger protein 15 (PHF 15) participates in chromatin remodelling (histone H3 acetylation, histone H4-K5 acetylation, histone H4-K8 acetylation, histone H4-K12 acetylation and histone H4-K16 acetylation). Proteins show molecular functions (metal-ion binding, protein binding, zinc-ion binding) and to localize in histone acetyltransferase complex. Putative protein interactions have been described (ING4, ING5 and MYST2) (Thierry-Mieg and Thierry-Mieg 2006). 'Protease, serine, 1 (trypsin 1)' (PRSS1) has a variety of roles in the body: food digestion, regulation of other proteins and modification of extracellular matrix. Evidence shows the importance of serine proteases in the nervous system as well. It has been shown that three serine proteases, thrombin, plasminogen activators and neuropsin, have functional roles in neural plasticity (Yoshida and Shiosaka 1999). Mannosidase alpha class 1A member 1 (MAN1A1) encodes a class I mammalian Golgi 1, 2-mannosidase which is a type II transmembrane protein. This protein catalyses the hydrolysis of three terminal mannose residues from peptide-bound Man(9)-GlcNAc(2) oligosaccharides and belongs to family 47 of glycosyl hydrolases (described in NCBI). A previous study (Zhang *et al.* 2013) found that three SNPs on OAR8 and OAR26 (two SNPs) had effect on SMW and were located 21,112–261,544 bp apart from the nearest known ovine genes (*RARB* and *OXSM*).

Yearling weight (YW)

We detected five significant SNPs on four chromosomes associated with YW. Three of these SNPs were located within the known ovine genes (*SYNE1*, *WAPAL* and *DAAM1*). OAR8_82161543 with genomewide significant effect on YW was located within QTL which has been reported to affect average daily gain (birth, 43 weeks) in Merino sheep (Raadsma *et al.* 2009). In this study, OAR8_82161543 was identified within Syne1 (spectrin repeat containing, nuclear envelope 1), which is associated with nuclear envelope in skeletal, cardiac, and smooth muscle cells (Apel *et al.* 2000). WAPL (wings apart-like homolog (*Drosophila*)) encodes a protein that regulates heterochromatin structure. Mutations of WAPL prevent the normal close apposition of sister chromatids in heterochromatin regions but do not appear to affect either heterochromatin condensation or chromosomal segregation (Verni *et al.* 2000).

In summary, in this study the GWAS detected 13 SNPs with chromosome-wide significance and one SNP with genomewide significance. These SNPs were reported for the first time. All significant SNPs detected in this study were within or close to known ovine genes. However, we recommend more studies to prove these genes as candidate genes for body weight.

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