RESEARCH NOTE

Genomewide association study to detect QTL for twinning rate in Baluchi sheep

MOHSEN GHOLIZADEH[1]*, GHODRAT RAHIMI-MIANJI[1], ARDESHIR NEJATI-JAVAREMI[2], DIRK JAN DE KONING[3] and ELISABETH JONAS[3]

[1]*Department of Animal Science, Laboratory for Molecular Genetics Animal Biotechnology, Faculty of Animal and Aquatic Science, Sari Agricultural Sciences and Natural Resources University, Sari, Iran*
[2]*Department of Animal Sciences, Faculty of Agronomy Sciences, College of Agriculture Natural Resources, University of Tehran, Karaj, Iran*
[3]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7023, 750 07 Uppsala, Sweden*

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Introduction

Twinning rate in sheep has great economic importance for farmers. A genomewide association study, using 42,416 single nucleotide polymorphisms (SNPs) was conducted to identify genomic regions affecting twinning rate in Baluchi sheep. Blood samples from a total of 96 sheep from two herds and data on their twinning rate during the first four parities were collected. Animals were genotyped using the Illumina Ovine SNP 50K BeadChip assay (Illumina, San Diego, USA). Association with twinning rate was tested using the software Plink ver. 1.06 (Purcell *et al*. 2007). Significant associations were identified for SNP on chromosomes 10 and 15 affecting total number of lambs across the four parities. Strongly suggestive quantitative trait loci (QTL) were also detected for twinning rate at different parities on these chromosomes. Further screening of these regions in validation studies will assist the identification of strong candidate genes for twinning rate in sheep.

Baluchi sheep are one of the most important sheep breeds in Iran, ∼ 30% of the total sheep population in Iran is from this breed. Baluchi sheep is especially well-adapted to the rather harsh conditions in the arid subtropical region of eastern Iran due to its breed-specific characteristics. This breed belongs to the fat-tailed sheep, its wool is coarse with white fleece and pigmented head and legs (Yazdi *et al*. 1997). Baluchi sheep are used for multiple purposes, including wool production and also play an important role in meat production (Tahmoorespur and Sheikhloo 2011). Therefore, the reproduction rate is one of the important selection criteria. Reproductive traits differ greatly across sheep breeds, but also between sheep in a single flock. Identification of ewes with higher twinning rate and more raised lambs per year is an important parameter for breeding and farming success. Phenotypic selection can be used to identify breeding stock with a higher probability of increased reproduction rate, however, the identification of causative mutations or markers closely associated with twinning rate could assist successful breeding for increased number of lambs born per parity. The study of QTL has been successful in identification of many loci on the sheep genome linked or associated with different traits, including reproduction (Hu *et al*. 2013). However, such studies were often based on the use of microsatellite markers and the screening of large populations had been time-consuming and identified regions often span tens of cM. A number of loci associated with reproduction in sheep have been previously identified (Galloway *et al*. 2000; Souza *et al*. 2001; Hanrahan *et al*. 2004). However as some of those loci might be population-specific and causative mutations might yet remain to be found, further screening of the genome to identify additional strong candidate genes should be done. Detection of large numbers of SNPs provides a critical resource for identifying specific loci contributing to trait variation (Johnston *et al*. 2011). With the development of sequencing and high-throughput genotyping techniques, the number of SNP markers available for association studies has dramatically increased (Sahana *et al*. 2010). A number of studies have already successfully identified QTL using genomewide SNP arrays also in sheep (Johnston *et al*. 2011; Zhao *et al*. 2011; Moradi *et al*. 2012). Demars *et al*. (2013) performed a genomewide association study (GWAS) and successfully identified genetic variants

*For correspondence. E-mail: m.gholizadeh@sanru.ac.ir.

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associated with the prolific phenotype in French Grivette and Polish Olkuska sheep populations near a functional candidate gene on the X chromosome. However, to be able to use such results for selection in the population of interest, verification studies should be performed in the population of interest. This will allow a further characterization of the genetic background and possibly identify strong genetic markers or even causative mutations for marker-assisted selection (MAS). The objective of our study was therefore to perform a GWAS for twinning rate in Baluchi sheep using the Ovine SNP50 BeadChip (Illumina).

Materials and methods

Animal husbandry

Sheep used in this study were kept on the Abbasabad Sheep Breeding Station, located in Mashhad in northeastern Iran. In our study the mating season for the sheep started in late summer (August) or early autumn (September) and included at most three estrous cycles. Lambing started in early February and ended in late March. Lambs were weighed and ear-tagged at birth, pedigree and birth records were registered separately for each lamb. Sheep had access to the available pasture during spring and summer, and grazed on wheat and barley stubble in autumn. The lambs were kept indoors during winter. A ration composed of wheat and barley straw, alfalfa hay, dry sugar beet pulp and concentrate was offered as feed supplement during winter and late pregnancy.

Animal samples

Blood samples were collected from two separate research flocks of Baluchi sheep along with data on twinning rate in the first four parities at the Abbasabad Sheep Breeding Station. More than 300 blood samples were collected in total and 96 samples with complete records over multiple parities were selected for further analysis. The 96 animals belonged to 43 half-sib families. Samples for genotyping were selected based on completeness of records, phenotype distribution (high and low twinning rates) and pedigree (lowest relatedness and high within-breed diversity).

Genotyping and data quality control

Genomic DNA was extracted from whole blood using a modified salting out protocol following Miller *et al*. (1988). DNA samples were diluted to 50 ng/*u*L for genotyping using the Illumina Ovine SNP 50K BeadChip Assay. Genotyping was performed using standard protocols (http://www.illumina.com) at the SciLife Lab in Uppsala, Sweden (http://www.genotyping.se). Quality control criteria including genotyping frequency >95%, minor allele frequency >0.05 and Hardy–Weinberg equilibrium ($P > 0.001$) were calculated using Plink ver. 1.06 (Purcell *et al*. 2007). Markers fulfilling these criteria were included for further analysis. Marker positions were used as published on the Illumina website (http://www.illumina.com).

Statistical analysis

Genomewide association analyses were carried out in Plink ver. 1.06 (Purcell *et al*. 2007). Genetic stratification and herd effect were included as confounding effects and fitted into the statistical analyses. Using Plink, an identical-by-state (IBS) correlation matrix for all individuals was calculated from which *n* dimensions were extracted using the multidimensional scaling (MDS) analysis, resulting in a matrix of *n* samples by *n* dimensions of eigenvalues. GWAS for twinning as well as total number of lambs across the four parities were performed with the first MDS component and herd effect as covariates. To control the family-wise error rate (FWER), a Bonferroni correction was used. The 5% genomewide threshold was equivalent to a nominal *P* value of 1.178×10^{-6} and the 5% chromosome-wide significance thresholds ranged from the point-wise *P* value of 1.078×10^{-5} on chromosome 1 to 6.96×10^{-5} on chromosome 21. GWAS results were visualized in a so-called 'Manhattan plot' using the 'ggplot2' package in R ver. 3.0.0 (http://www.r-project.org). For each of the significant SNPs, the additive genetic effects were calculated as half of the difference of the least square means of the two homozygous genotypes. The dominance effect was also estimated as the deviation of the least square mean of the heterozygotes from the average of the least square means of the two homozygous genotypes.

Study of genes and QTLs in candidate regions

Different databases were screened to discover if any of the regions identified by the association analysis contained strong candidate gene. After obtaining the sheep genome sequence of the significantly associated candidate regions using the CSIRO browser (ver. 3.1, as on October 2012, http://www.livestockgenomics.csiro.au), a BLAST search was performed using the bovine UCSC Genome Browser (October 2007, Baylor 4.0/bosTau4) to identify genes already mapped to the bovine genome. We also searched for previously published loci using the QTL database (http://www.animalgenome.org/QTLdb) to further verify the identified candidate regions with previously published QTL in cattle.

Results and discussion

Genotyping was performed on 96 Baluchi sheep using the Illumina Ovine SNP 50K BeadChip. After quality control, 42,416 SNP markers remained for further analysis. A number of significant and suggestive associated markers were identified in the different analysis, the GWAS showed three different QTL regions on two chromosomes. Statistical evidence for the two genomewide significant QTL for total number of lambs across all parities on chromosomes 10 (*P* value = 3.6×10^{-5}) and 15 (*P* value = 3.7×10^{-5}) is visualized in figure 1. For separate parities (figures 1–4 in electronic supplementary material at http://www.ias.ac.in/jgenet), both loci were chromosome-wide significant, but did not exceed the

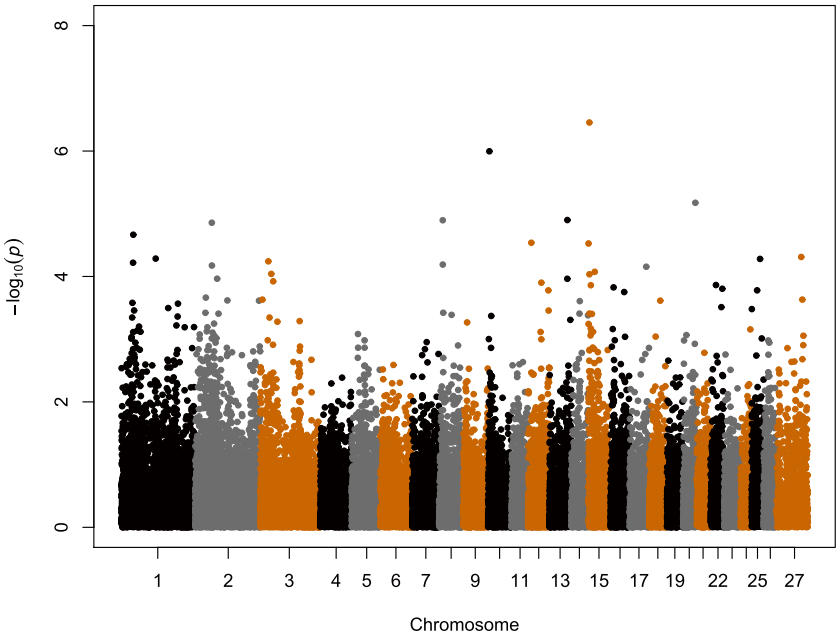

Figure 1. Manhattan plot of the results from the genomewide association analysis for total number of lambs across all four parities.

genomewide significance level (table 1). Both QTL seem to act in a dominant fashion. The QTL on chromosome 15 had the strongest effect with a difference of ∼1 lamb between the alternative homozygotes for the most significant SNP (table 1). The QTL on chromosome 10 had a smaller effect of ∼0.3 lambs between alternative homozygotes accumulating to a difference of 1 lamb across the four parities (table 1). The QTL detected in our study appear to be novel in sheep as none of the previous studies have reported significant loci within the same regions. In sheep, three prolificacy loci have been discovered on chromosome 6 (Souza *et al*. 2001) chromosome 5 (Hanrahan *et al*. 2004) and chromosome X (Galloway *et al*. 2000; Hanrahan *et al*. 2004). Lack of genomewide significance may be evidence of a complex genetic nature for twinning in sheep. Litter size is a complex trait that is a function of ovulation rate, embryo viability and uterine capacity (Bennett and Leymaster 1989). A simulation model suggested that litter size is strongly dependent on ovulation rate and uterine capacity in pigs (Bennett and Leymaster 1989). It is likely that the twinning rate in sheep is not only dependent on ovulation rate but also an uterine capacity. This would implement that the embryo mortality would be higher in ewes with higher ovulation rate if the uterine capacity is limited. As such, ewes with a higher ovulation rate would show no difference in final lambing rates compared to ewes with lower ovulation rates (Waldron and Thomas 1992). Identified loci might therefore affect either of the two traits, total ovulation rate or uterine capacity. However, as abortions are occurring often early and might not always be observed, a clear separation of the two traits might be difficult.

Lack of genomewide significance at the individual SNP level for twinning rate may also indicate that more samples are required to identify stronger signals from a GWAS for reproduction traits in sheep. However, significantly associated markers were previously reported on the ovine X chromosome in a study of 104 individuals (Demars *et al.* 2013). The number of animals in the present study together with the repeated phenotypic measurement might therefore be

Table 1. Significantly associated SNP with twinning rate identified in this study with their estimates of the additive and dominance effects (in number of lambs) with their standard errors and error probabilities.

Trait	Parity	CHR	SNP	Position (in base pairs)	*P* value	Additive	*P*	Dominance	*P*
TW	1	10	OAR10_4976585.1	6730970	5.88E-05	0.16 ± 0.08	0.07	0.17 ± 0.1	0.11
	2	10	OAR10_4976585.1	6730970	2.90E-05	0.14 ± 0.08	0.11	0.14 ± 0.1	0.17
	3	15	OAR15_4302920.1	4912198	2.12E-05	0.55 ± 0.1	<0.0001	0.49 ± 0.12	0.002
	4	10	OAR10_4976585.1	6730970	5.88E-05	0.17 ± 0.08	0.07	0.12 ± 0.08	0.24
TNB	1–4	15	s71038.1	4776717	3.51E-07	1.27 ± 0.36	0.0007	1.38 ± 0.49	0.002
		10	OAR10_4976585.1	6730970	1.01E-06	0.47 ± 0.31	0.13	0.57 ± 0.39	0.15

TW, twinning; TNB, total number of lambs across four parities; CHR, chromosome; SE, standard error; *P*, error probability.

Table 2. QTLs previously published in cattle in regions showing significant associations in our study.

Trait	SNP[a]	Position (bovine)[b]	Position (ovine)[b]	Gene[c]	Accession number	Reference
Twinning	OAR15_4976585	12:5097625–5097934	10:6780796–6710878	PCDH17 5,086,499–5,200,843	NP_001192609	Lien *et al.* 2000
Inseminations per conception	OAR15_4302920	15:4270442–4270956	15:4889401–4974368	PDGF-D 15: 4,501,435–4,788,300 DDI1 15: 4,642,030–4,644,091	NP_001077175 NP_001071488	Schulman *et al.* 2008

[a]Significant SNP in our study; [b]relative position (chromosome and base pairs) on the bovine and ovine genome; [c]protocadherin-17 precursor (PCDH17); platelet-derived growth factor D (PDGF-D); DNA-damage inducible 1(DDI1).

adequate to detect the QTL with moderate to large effects, which are most relevant for MAS.

Orthologous regions between sheep and cattle together with genes and QTL identified in the bovine genome in regions containing the most significantly associated markers are presented in table 2. The results showed that only relatively few genes have been mapped to these regions until now and no gene associated with twinning rate has been reported in bovine orthologous regions. However, QTL published in the database (QTLdb) affecting twining rate and also other reproductive traits suggest the relevance of the identified regions. Further analyses using a larger sheep population might be useful to verify the associated regions. The identification of positional and/or functional candidate genes as targeted by comparative mapping with results published for cattle populations might give further evidence for the importance of the identified regions in our study. Strong candidate genes could then be screened for possible causative mutations and trait specification being linked to the two possible restrictions of higher lambing rates; total ovulation rate and uterine capacity. However, beside the limited number of animals from a single population in our study, this approach studying repeated measurements from different parities appears to be useful as a starting point to identify (population-specific) markers for twinning rate in sheep.

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