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Quantitative trait loci affecting pre-weaning weights in Baluchi sheep

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ABSTRACT

The objective of this study was to identify QTL affecting birth weight, 30, 60, 90 days weight in Baluchi sheep using data from half sib families and microsattelite in ovine chromosome (OAR1).The population study consisted of 434 Baluchi fat-tailed Iranian lambs from 11 half sib families, with progeny per family ranging from 29–54 individuals. Records on day of birth, sex, type of birth, age of dam and weight measurements at birth and 1 month intervals after birth up to weaning (90 days), were collected. Animals genotyped based on the panel of 8 microsattelite markers were selected based on positional candidate gene in OAR1. Lambs were genotyped for informative microsattelite markers, markers that were heterozygous in their sire, on chromosome 1. Live weights measured at birth and 1 month interval to weaning (Bw, 30W, 60W, 90W) were treated as separate traits. Fixed effects for all live weights were year, sex, type of birth, age of dam. The day of birth or age at time of measurement was fitted as covariate for each trait. QTL analyses were conducted using a univariate multi-marker for half sib families, for each trait by interval mapping approach. Genome wide permutation indicated significant QTL for birth weight at closest to marker BMS2572 at 204.8 cM, for 30 and 60 days weights at the 213 and 189 cM, respectively, between the markers BMS2321 and BM7145 and for 90 days weight at 186.8 cM between the markers BMS2321 and BMS2572. The proportions of phenotypic variance explained by QTL were 18, 12, 15 and 21% for birth, 30, 60 and 90 days weight at its maximum. The absolute size of distinct QTL effects varied according to the trait means and QTL effects increased with age. Because of this reason traits were highly correlated so detection of QTL live weight as single trait fails to capture correlations between components.

Key words: QTL, Quantitative trait loci, Pre weaning weight, Baluchi sheep

The pre-weaning growth rate of lambs is an important economic trait, which is controlled by complex environmental and direct genetic effects. Heritability studies of growth-related traits indicated that there are genetic mechanisms that partially control these traits (Cheng *et al.* 2011). Therefore growth traits have been widely studied in animal models (Safari *et al.* 2005). A good understanding of mechanisms that control growth trait will benefit both meat production and human health.

Identifying genes affecting quantitative traits (QTL) of economic importance has the potential to significantly increase the rate of genetic improvement through the use of marker assisted selection (Spelman and Bovenhuis 1998). In addition, genomic research and the identification of QTL help improve our understanding of the underling biology of a specific trait.

Studies in livestock reported QTL association with birth-weight (Davis *et al.*1998, Stone *et al.* 1999, Stearns *et al.* 2005, Hadjipavlou *et al.* 2009, Margawati *et al.* 2006, Esmailzadeh, 2010) and 20 week weight (MacRae *et al.*2005, Walling *et al.* 2004).

The objective of this study was to identify QTL affecting birth weight, 30, 60, 90 days weights in Baluchi sheep using data from half sib families and candidate position on chromosome 1.

MATERIALS AND METHODS

Animals and trait measured: The population of study consisted of 434 Baluchi fat-tailed Iranian lambs from 11 half sib families, with a number of progeny per family ranging from 29–54 individuals (Table 1). The animals were recorded for 2 years (2008, 2009). Records and information on day of birth, sex, type of birth, age of dam and weight measurements at birth and one month intervals after birth up to weaning (90 days), were collected.

Genotyping: DNA was extracted from blood samples (Boom *et al.* 1990) using DNA extraction kit. The quality and quantity of DNA were measured by using nanodrop machine.

A panel of 8 microsattelite markers (Table 2) was selected based on positional candidate gene studies on chromosome

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Table 1. least square mean of birth weight, 30, 60 and 90 days weight, number of progeny in family studied

Families	1	2	3	4	5	6	7	8	9	10	11	mean
No of progeny	58	51	43	36	45	34	29	30	34	36	38	37
Bw[a]	4.04	4.36	4.04	3.7	4.3	4.1	4.3	4.12	4.23	4.03	3.88	4.12
	±0.6	±0.7	±0.8	±0.7	±0.7	±0.5	±0.8	±0.6	±0.6	±0.5	±0.7	±0.7
30W	10.81	10.39	9.63±	9.77±	11.22	10.8	11.41	11.07	11.47	11.22	10.68	10.69
	±2.6	±2.7	2.4	2.1	±3	±2.5	±1.76	±2.5	±2.7	±3.1	±2.6	±2.5
60W	16.57	16.23	14.83	15.03	16.81	16.11	17.06	16.53	17.23	16.39	15.97	16.2
	±3.7	±3.7	±4	±3.6	±3.9	±3.9	±3.22	±3.5	±3.9	±4.9	±4.3	±3.91
90W	20.75	20.68	18.97	19.39	21.7	20.82	22.14	20.77	21.44	20.78	20.28	20.55
	±4.6	±4.5	±4.5	±4.9	±5.1	±4.5	±2.5	±3.6	±3.9	±5.2	±4.7	±4.5

a, traits in this study birth weight (Bw), 30 days weight (30W), 60 days (60W), 90 days weight (90W).

1 ovine, MacRae *et al.* (2005) and Walling *et al.* (2004). The genomic intervals containing trans-ferrin and growth hormone factor 1 located on OAR1 were chosen based on Raadsma *et al.* (2009) and from genetic DNA library available on the Australian Sheep Gene mapping website (http: // www.thearkdb.org/arkdb).

Lambs were genotyped for informative microsattelite markers, i.e. markers that were heterozygous in their sires, located on chromosome 1 (Table 1). PCR amplification conditions were as 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30s and extraction at 72°C for 30s and final extraction at 72°C for 5 min.The amplified fragment were electrophoreses on 8% poly-acryl amide gels with 100 to 150 V of running time voltages. Gels were detected by silver staining. The genotype of each animal for 8 different loci was recorded by direct counting.

Phenotype and treatment: Live weights measured at birth and 1 month intervals up to weaning (Bw, 30W, 60W, 90W) were treated as separate traits. GLM procedure of SAS 9.2 was applied to each trait to identify significant factors that need to be accounted for in the QTL mapping model. Fixed effects for all live weights were year, sex, type of birth and age of dam. The day of birth or age at time of measurement was fitted as covariate in analysis of all individual traits.

QTL analysis: QTL analyses were conducted using a univariate multi-marker approach for half sib families, as described by Knott *et al.* (1996). GridQTL (Seaton *et al.* 2006), a web-based QTL analysis program, was used to identify QTL associated with each traits by interval mapping. The QTL model included the fixed effects of year, sex, type of birth, age of dam, and age of lamb at time of measurement as covariate for each trait. The probability of inheriting a particular sire allele was calculated at 1cM intervals for each offspring, conditional on the marker genotypes of individual and its sire. Subsequently, the phenotype of each trait was regressed on the conditional probability of the offspring genotype inheritance for given position.

Across family analysis was applied to calculate the estimation of putative QTL effect for each sire included in the analysis for the genomic region under investigation. This was done by nesting regression within sires. This was done because all sires would not be heterozygous for all QTL and the linkage between the QTL and the sire haplotype might vary from sire to sire.

For each regression, F-ratio was provided by GridQTL in which the full model including the significant fixed effects, covariate and QTL effect was compared with the same model without QTL effect. The chromosomal location with the largest F-ratio was considered to be the best estimation of position for a QTL for each trait. The percentage of phenotypic variation accounted for by a QTL position was computed as $4*(1-RMSQ_{full}/RMSQ_{reduced})$, where, $RMSQ_{full}$ and $RMSQ_{reduced}$ are residual mean squares of the model with QTL and without QTL effect, respectively (Knott *et al.* 1996).

An estimate of overall QTL effect was obtained by calculating the average of absolute values of QTL allelic substitution effect across family for which QTL was significant. Genome-wide significance levels (1% and 5%) were determined by the genome-wide permutation procedure (Churchill and Doerge 1994) implemented in GridQTL, using 10,000 permutations. Confidence interval for the location parameter obtained by bootstrapping (Visscher *et al.* 1996).

RESULTS AND DISCUSSION

Least square means of pre-weaning weights are presented in Table 2. The analysis of variance showed that flock, sex, type of birth and age of dam were significant sources of variation in pre-weaning weights (P<0.01). Year of birth was not significant on pre-weaning weights. Mean performance of animals were consistent with previously published values (Yazdi *et al.*1998).

All sires revealed significant QTL for birth weight at closest to marker BMS2572 at 204.8 cM, (P<0.01), with 2.15 (kg) QTL effect as the average of absolute values across families in which the QTL was significant (Table 3). The QTL for 30 and 60 days weights were significant (P<0.05) at the 213 and 189 cM, respectively, between the markers BMS2321 and BM7145. For 90 days weight QTL was estimated to be at 186.8 cM between the markers BMS2321 and BMS2572 with significant effect (P<0.01; Table 3).

Table 2. Marker position, number of heterozygous sire, proportion of hetrozygosity and number of allele in choromosome 1

Marker	BM6465	BMS1636	BM4129	BMS2321	BMS2572	BM7145	BMS4008	BM8246
Position[a]	80.8	91.9	101.9	154.1	209.4	229	231.1	236.6
HS[b]	7	6	6	4	8	8	10	5
He(%)[c]	53	44	42	30	39	61	76	39
Number allel	8	6	5	7	7	4	7	5

a, based on positional from genetic DNA library available on the Australian sheep gene mapping website (http: //www.thearkdb.org/arkdb);.b Number of heterozygous sire; c, proportion of hetrozygosity (%) in each marker.

Table 3. Most likely position, F-static, allelic effect of detected QTL for birth weight, 30, 60 and 90days weights in Baluchi sheep

Trait[a]	Position (cM)[b]	Families[c]	Effect [d]	F-value[e]	%Vp by QTL[f]	Marker interval[g]	CI[h]
Bw	204.8	3, 9	1.9	2.39 (1.84–2.33)**	18	BMS2572	2–150
30W	213.8	5, 8, 9	3.26	2.1 (1.8–2.26)*	12	BM7145	0–153
60W	189.8	3, 8	7.29	2.08 (1.8–2.25)*	15	BMS2321–BMS2572	0–153
90W	186.8	3, 8	9.7	2.46 (1.82–2.3)**	21	BMS2321-BMS2572	0–153

a, traits in this study birth weight (Bw), 30 days weight (30W), 60days (60W), 90 days weight (90W); b, QTL location based on first marker position at 80.8cM;c, families within which a QTL effect was significant;d, QTL allelic substitution effect as the average of absolute values across families for which the QTL was significant;e, chromosome wide F-static as determined by permutation test, values for P<0.05 and P<0.01 are given in parentheses;f, percentage of phenotypic variation accounted for by a QTL position was computed as $4*(1-RMSQ_{full}/RMSQ_{reduced})$ where $RMSQ_{full}$ and $RMSQ_{reduced}$ are residual mean squares of the model with QTL and without QTL effect respectively (Knott *et al.* 1996); g, QTL location confidence interval as determined by bootstrapping;h, confidence interval.

Confidence intervals (0.95) for the location parameter are given in Table 3 for those QTL attaining 0.05 chromosome wide significance across the families. For the birth weight, 30, 60 and 90 days weights QTL region about 87, 96, 89 and 93 cM (averaged over the male and female map) is spanned by this 0.95 confidence interval which are large proportion of the total investigated intervals 167, 176, 169 and 173 cM, respectively.

F-ratio profile and the frequency distribution of the bootstrap results for the best locations of QTL for each trait are given in Fig 1. This distribution for birth weight, 30, 60, 90 days weights was similar to the F-ratio profile, with a large number of the replicates giving a maximum test static at 204, 213, 189 and 186 cM respectively. The QTL that were detected for different traits were localized to the same chromosomal region.

Chromosome 1 was chosen in the study because of the presence of the growth hormone gene, which reportedly has association with growth traits (Kmiec 1999). QTL analysis for pre-weaning weight showed that there was a chance of QTL for birth weight, 30, 60 and 90 days weights at 167, 176, 169 and 173 cM, respectively. Compared with previous QTL mapping studies in the sheep, birth weight QTL position identified in our study were similar to QTL positions that had been previously observed. This study indicated the effect of candidate gene for birth weight at the region of flanking marker BMS2572 was very close to the largest QTL identified by Esmailizadeh (2010) in Kermani sheep on chromosome 1.

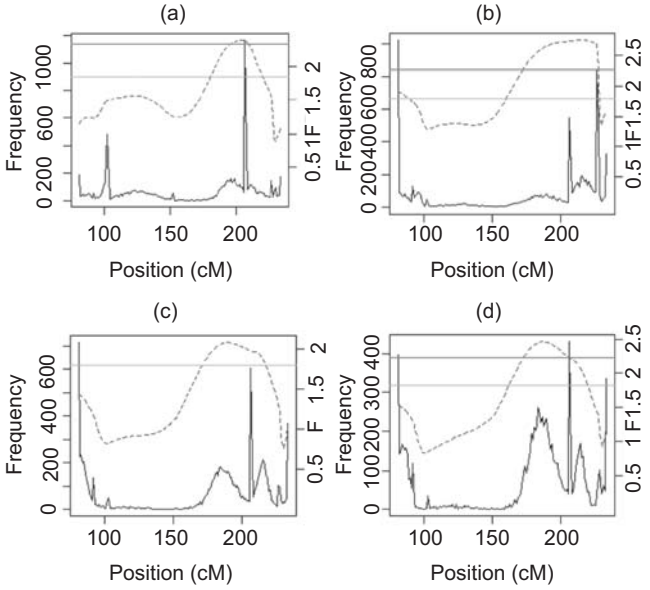

Fig. 1. F-value profile obtained from across family analysis (dashed line), the best location estimates from the bootstrap analysis (solid line), lower (gry) and upper (green) horizontal line represent 5 and 1% chromosome wide significant levels of linkage for birth weight, 30, 60, 90 days weight at a, b, c, d, respectively.

In addition these regions on chromosomes 1, that contain QTL in our study for 30, 60 and 90 days weight were not shown to be associated with body weight traits in sheep before although other regions on the same chromosomes are known to be having body weight QTL.

MacRe *et al.* (2005) have analyses results of partial scans on selected autosomal and candidate region on OAR (1,2,3,5,6 and 11) in Suffolk and Texel commercial sheep populations. They identified QTL for body weight at 8 and 20 weeks of age on OAR1. Walling *et al.* (2004) have revealed suggestive QTL for body weight at 20 weeks of age on OAR1 and OAR3. Furthermore, in cattle, birth weight QTL was reported in a number of independent studies (Stone *et al.*1999, Esmailzadeh 2006), on bovine chromosome 1 (BTA1) which is homologues to OAR1.

The estimation QTL effects for each trait (Table 3) suggested that the size of the detection QTL effect in terms of the proportion of phenotypic variance explained by QTL at its maximum. However, the absolute size of distinct QTL effects varied according to the trait means. i.e. live weight QTL effects increased with age. One reason might be that traits were highly correlated so detection QTL live weight as single traits fails to capture the correlations between the components.

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