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Co-Segregation of Quantitative Trait Loci (QTL) Affecting Pre-Weaning Traits for Fat-Tailed Ghezal Sheep in Chromosome 1

Research Article

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ABSTRACT

This study exploited the co-segregation of quantitative trait loci (QTL) affecting pre-weaning traits in Ghezal sheep. Two half-sib families (n=71) were genotyped for 8 informative microsatellite markers covering chromosome 1. Data for production traits (birth weight (BW), weaning weight (WW), average dairy gains (ADG) and Kleiber ratio (KBR) were collected. Investigated microsatellite loci were successfully amplified in progenies and allele numbers per locus ranged from 2 (CSSM11) to 10 (MAF109). Two models used for estimation of QTL effect were across families and individual families. QTL mapping were conducted using online GridQTL. The results show that two the QTL retained significance (P≤0.01) for BW and KRB at the region of flanking markers CSSM019-CSSM032 and BM1312 respectively. Further studies will be useful using more families, animals and chromosome number for identification of co-segregation of QTL affecting pre-weaning traits in Ghezal sheep.

KEY WORDS Ghezal sheep, half sib, microsatellites, QTL mapping.

INTRODUCTION

Growth during the pre-weaning, in particular, is the most key factor in rearing sheep for determining of more profit to the farmer. There is a growing body of literature that recognizes the importance of early expressed traits which influenced not only by additive genetic contribution, but also by ewe genetic and non-genetic effects such as dam age, deficiency of nutrients in intrauterine and placental barrier may play role for birth weight (BW) condition (Gardner *et al.* 2007). Understanding of genetic architecture of pre-weaning trait plays a crucial role and insights into the essential knowledge about this period life. Such information may help genetic improvement by the accurate selection.

Early stage of life in livestock has complexity due to weak immune system and high susceptibility of neonate to environmental pathogens. BW is associated with post weaning growth traits in general as well as the mature market weight (Boligon *et al.* 2009). The weaning weight (WW) is used as a criterion to select animals for further breeding (Guidolin *et al.* 2012).

Low lamb BW has significant negative correlation with high neonatal mortality rate and the other side of the coin, high lamb BW increased frequency of injury or death of ewe and lamb during conception (dystocia) and need veterinary assistant, or veterinary technician (Alexander, 1974). A considerable amount of literature has been published on relationship between lamb BW and lamb mortality and survivability during weaning (Fogarty *et al.* 1992; Hatcher *et al.* 2009). Data from several studies in different sheep breeds demonstrated that direct and maternal heritability rate obtained of BW have been highlighted within 0.15–0.21 and 0.18–0.24 ranges, respectively (Safari *et al.* 2005).

Up to now, great attention has been paid to identification of the casual mutation within variety of major genes for growth trait in different sheep breed. In this regards, DNA technology offering new powerful tools for understanding of genetic architecture of economical trait in livestock as well as sheep (Andersen *et al.* 2004).

Microsatellite markers are most powerful tools for discovery of polymorphism within genome due to following advantageous: co-dominate nature, high distribution, high number of alleles, automated genotypes scoring and specific computer tools for interpretation of data. Numerous applicability of microsatellite markers in livestock was applied such as genetic diversity, parentage test, linkage maps and QTL mapping. The ovine linkage map consisted of 1374 markers representing 1333 loci arranged 3580 cM and in overall, 2325 Sheep QTLs for 251 different traits according to 158 publications was reported in animal QTL database website.

It is still not known which QTL significantly control both BW and WW in indigenous sheep. Within this context, in this paper, we argued about QTL simultaneously influencing BW and WW in Ghezal fat tailed sheep.

MATERIALS AND METHODS

Animals and sampling

In overall, 71 individuals from two Half Sibs families were taken from Meyandoab breeding station. The system of mating within herds was performed according to natural service and during of mating season, first age weight and body conformation of each candidate ram was monitored and then candidate sire introduced to 15-20 ewes after heat detection for service. After lambing season, ear tag identification system was used for newborn lambs and those sire with high number of offspring was used for establishment of paternal half sibs families for QTL analysis.

Two half-sib families (n=71) were used to analyze QTL for four production traits [BW, WW, average daily gain (ADG), Kleiber ratio (KBR)]. Following formula was used for adjustment of different lamb weaning age for 90 days:

Acual, WW= (Acual, WW-BW) / (Acual, Weaning age (days)) × (90+ BW_0)

The secondary data (ADG) was calculated for ADG1 (birth until 90 days using following formula:

ADG (g/day)= (WW-BW) / (weaning age) × 1000

Kleiber ratio: Kleiber= rate of growth / mass of body$^{0.75}$

Molecular analysis

In overall, 71 individuals from two paternal half sib's families were selected from whole population.

DNA extraction, PCR reactions and samples genotyping

DNA was extracted from blood, according to Samadi Shams conventional protocol (Samadi Shams *et al.* 2011). A nonodrop tool was used for measurement of extracted DNA purity according to OD 260/280 nm ratios.

Eight polymorphic microsatellite markers located in chromosome one were used for the genotyping. The marker spacing on the chromosomal map was between 5 to 40 cM. Priority for selection of candidate microsatellite markers was according to reported polymorphism information content (PIC), sharp electrophoresis pattern and exhibition of high hetrozygosity for each sire for traceability of alternative alleles within their offspring. Table 1 shows summary of investigated loci, motif, allelic size, primer sequence in this study.

Electrophoresis method in 4% metaphor gels at 65 V for 3 or 2 h, depending on the expected allele sizes was applied for polymerase chain reaction (PCR) products. 25 bp ladders of molecular weight markers were employed for estimation of allele size range within each specific SSR locus. That microsatellite indicated uninformative genotype was excluded from further statistical interpretation.

PCR amplifications were carried out in 25 µL reactions containing: 1 µL of genomic DNA template, 2.5 µL of 10x PCR buffer, 0.5 µL dNTPs (10 µM stock), 1.5 µL $MgCl_2$ (25 µM stock), 0.2 µL each primer (25 µM stock), 0.5 µL Taq- Dream Taq (1.25 units/mL) and 18.6 µL of de-ionized water (ddH_2o). All reactions were run on either an Epperndorf Master cycler® X50 thermal cycler.

A touchdown nucleic acid amplification protocol was carried out for minimizing stutter band and genotyping error as following detail: initial denaturation (94 ˚C-6 min); (b) 1 cycles of denaturation (94 ˚C-45 s), annealing (68 ˚C-50 s) and extension (72 ˚C-50 s); (c) 1 cycles of denaturation (94 ˚C-45 s), annealing (66 ˚C-50 s) and extension (72 ˚C-50 s); (d) 1 cycles of denaturation (94 ˚C-45 s), annealing (64 ˚C-50 s) and extension (72 ˚C-50 s); (e) 1 cycles of denaturation (94 ˚C-45 s), annealing (62 ˚C-50 s) and extension (72 ˚C-50 s); (f) 1 cycles of denaturation (94 ˚C-45 s), annealing (60 ˚C-50 s) and extension (72 ˚C-50 s); (g) 25 cycles of denaturation (94 ˚C-45 s), annealing (58 ˚C-50 s) and extension (72 ˚C-50 s) and final extension 72 ˚C for 5 mins.

Table 1 Summary of general characteristics of primers and genes studied in this research

Locus	Primer sequence	Allelic size (bp)	Motif sequence
MAF64	AATAGACCATTCAGAGAAACGTTGAC CTCATGGAATCAGACAAAAGGTAGG	109-141	(TG)13
ILSTS004	CTTAAAATCTGTCTTTCTTCC TAGTGTGTATTAGGTTTCTCC	90-106 100	(CA)16
CSSM004	ATGCGTCCTAGAAACTTGAGATTG GAAATCATCTGGTCATTATCAGTG	183-186 196-220	(GT)10(TA)5
CSSM11	TGTTTTAAGCCACCCAATTATTTG TTGTCAGCAACTTCTTGTATCTTT	173-197	(CA)3.CG.(CA)12
MAF109	GGAAGATTAGAACTTTCATATATCTTTAAACTC AATTGAATTTGAAGTGTATATGCCTAAATGC	157-195	(CT)7TT(CT)14(AT)6GT (AT)8
CSSM019	TTGTCAGCAACTTCTTGTATTCTTT TGTTTTAAGCCCACCCAATTATTTG	137-155 143-159	(TG)18
CSSM032	TTATTTTCAGTGTTTCTAGAAAAC TATAATATTGCTATCTGGAAATCC	206-220 226	(CA)19
INRA011	CGAGTTTCTTTCCTCGTGGTAGGC GCTCGGCACATCTTCCTTAGCAAC	203-215	(AC)8AT(AC)9

Statistical analysis

Two models were used for estimation of QTL effect: across families, individual families for multiple marker analysis according to Knott proposed regression procedure (Knott *et al.* 1996). QTL mapping were conducted using online GridQTL using 1-cM intervals for marker genotypes within chromosome. Generate F-ratios for position of each putative QTL was carried out during fixation of presumption of analysis. Lander and Botstein method for estimation of the LOD drop-off method and confidence intervals (CI) of each QTL locations was tested (Lander and Botstein, 1989). Displaying of cutoff for suggestive and significant thresholds of identified QTL was indicated according to Lander and Kruglyak (1995) report and following of permutation test (Churchill and Doerge, 1994). Construction of parental half sib families allowed offspring receives a dams originated random alleles at the marker locus. The QTL statistical analysis for HF design was according to Soller and Genizi (1978) linear model with following detail:

$$Y_{ijk} = \mu + S_i + M_{ij} + e_{ijk}$$

Where:

Y_{ijk}: phenotypic value for the k-th offspring of the i-th sire which receive j-th marker allele.

μ: population mean for the trait.

S_i: effect of the i-th sire (1, 2).

M_{ij}: effect of the j-th marker allele of the i-th sire.

e_{ijk}: residual effect.

Regression model to find the QTL position was employed according to Haley and Knott (1992) suggestion within candidate the chromosome.

$$y = \mu + \alpha X1 + \beta X2 + e$$

Where:

y: observed phenotype

X1= P (QQ | Mi)–P (qq | Mi)

X2= P (Qq | Mi)

X_1 and X_2: probabilities for QTL genotypes.

α and β: term in regression coefficients indicated the difference between the homozygote QTL genotypes and the QTL dominance effect, respectively.

They suggested formula for approximate likelihood ratio test statistics was indicated as follow:

$$LR = n\ln(SSE_{reduced}) / (SSE_{full}) = n\ln(1-r^2)$$

r^2: usual R-square for justification of percentage of variance.

RESULTS AND DISCUSSION

PCR-SSR and animal genotyping

Figure 1 shows quality of expected genomic DNA and amplification of PCR products in different investigated loci.

Investigated microsatellite loci were successfully amplified in progenies and allele numbers per locus ranged from 2 (CSSM11) to 10 (MAF109). Figure 2 also shows observed allele size and individual actual allele frequency in different investigated microsatellite markers in this study.

The information content of an individual marker is the proportion of animals in which the allele inherited from the sire can be unambiguously identified. Average information content across the two families and all of the investigated intervals along chromosome 1 ranging from 0.86 to 1 (Figure 3). Figure 4 shows permutation test results for the individual families and across families' analyses on 5% and 1% significant levels obtained for measured traits.

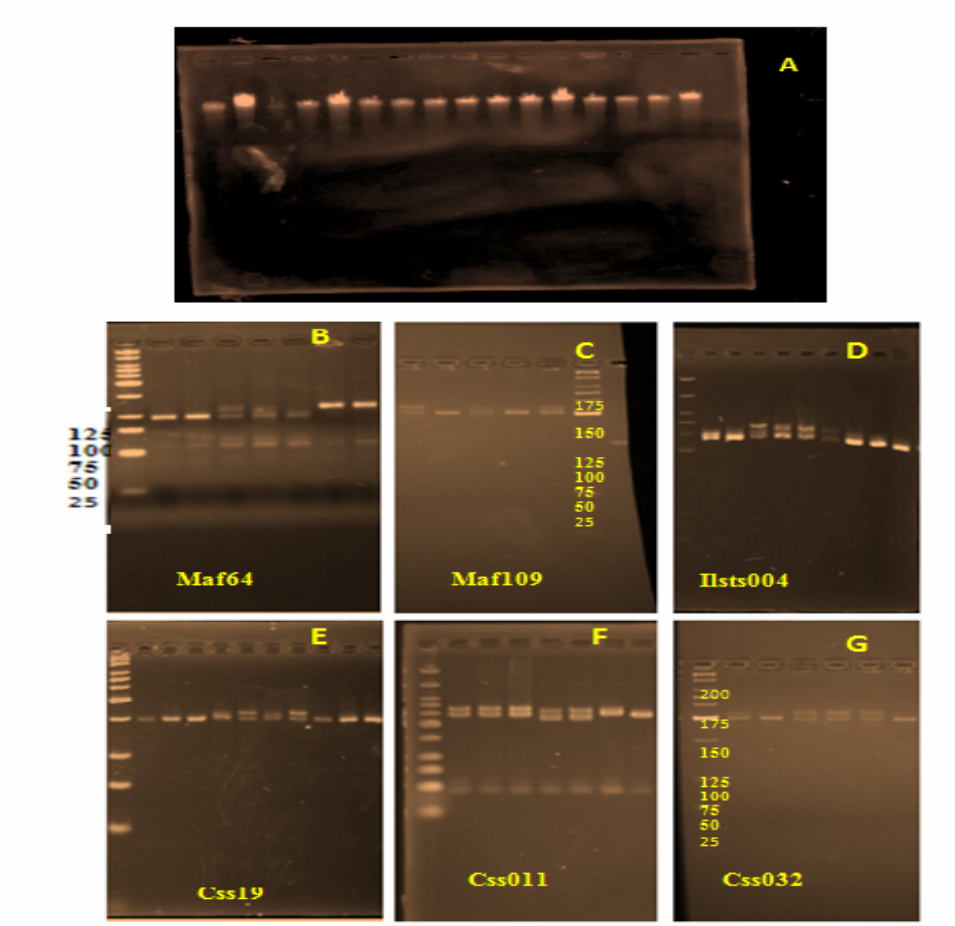

Figure 1 Quality of expected genomic DNA and amplification of PCR products in different investigated loci: 25 bp Ladder marker

Two the QTL retained significance (P≤0.01) for BW and KRB at the region of flanking markers CSSM019-CSSM032 and BM1312, respectively. Table 2 shows summary of significant QTL from individual family analyses.

Significant output of present reports was observation of co-segregation of QTL for ADG and Keliber ratio shows between measured per weaning traits in Ghezal fat tailed sheep in chromosome 1 were identified in 654 and 624 CM in family number 1 (chromosome-wide significance of (P<0.01).

Figures 5 and 6 shows F-statistic curves resulted from the QTL analysis of individual half-sib families and across families' analysis on chromosomes 1 of sheep.

Understanding of genetic architecture and casual mutation of candidate gene responsible for growth may help for respond to directional selection. Popularity of sheep meat and consumer preference for this species is unbelievable due to religious and cultural perspective. Therefore, motivation of genetic researcher is strong to focus on growth trait in indigenous sheep breed.

BW and WW are the early stage of growth characters and main key impacts on lamb survivability and growth performance traits. BW and WW of Ghezal sheep under different management systems have been reported and vary from 3 kg to 4.21 kg and 19.79 kg to 25.83 (Baneh, 2009).

Growth trait was assumed with moderate heritability and pre-weaning growth trait correlated positively with market live weight of animals. Paternal half Sibs families QTL design is based on capability for search of Mendelian inheritance of heterozygote genotype rams pattern to their offspring during tracing of pedigree structure (Haley and Knott, 1992).

The outputs of this report and identified QTL were comparable with other similar work on chromosome 1. Two QTLs retained significance (P≤0.01) for BW and KRB at the region of flanking markers CSSM019-CSSM032 and BM1312 respectively.

Based on high homology rate and cross species studied on linkage map of three bovines, ovine and caprine species, comparison of QTL mapping across species are possible.

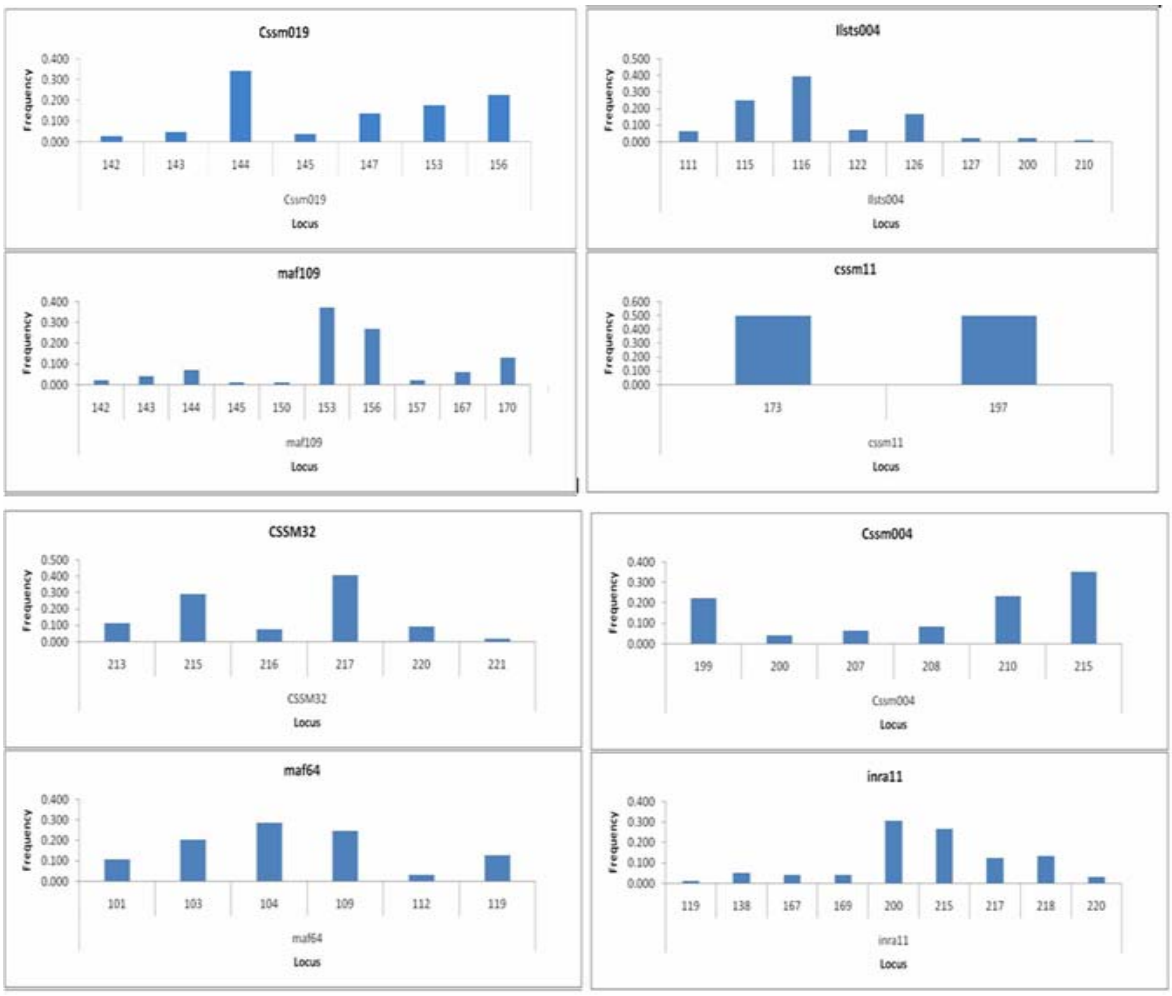

Figure 2 Observed allele size and individual actual allele frequency in different investigated microsatellite markers in this study

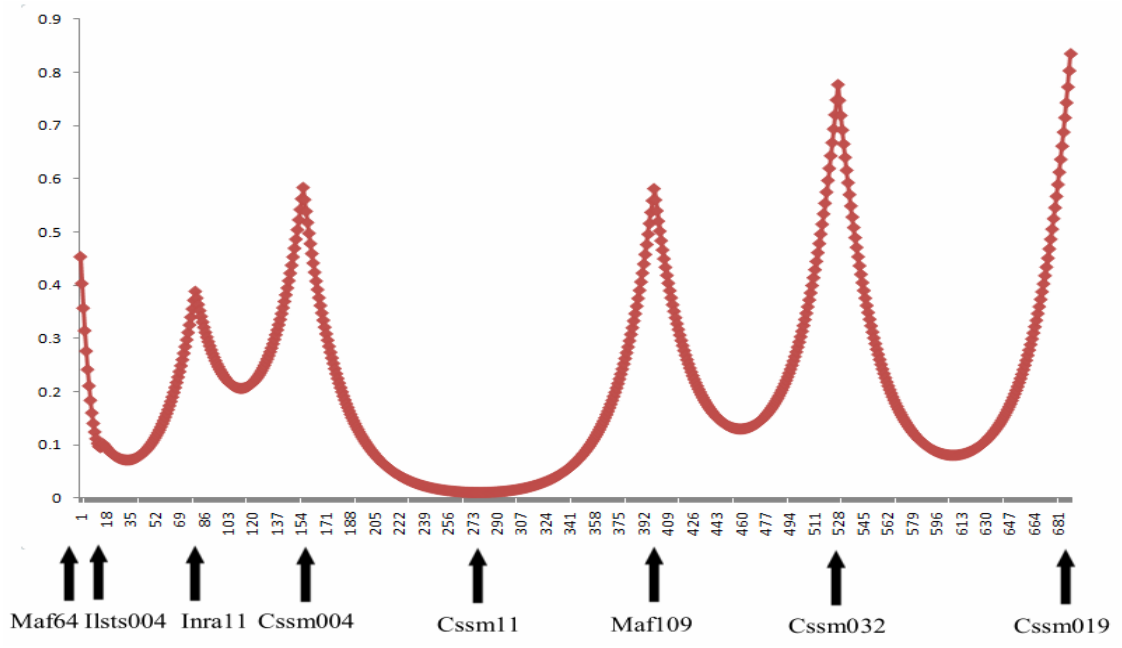

Figure 3 Information content across chromosome 1 in half-sib families of Ghezal sheep

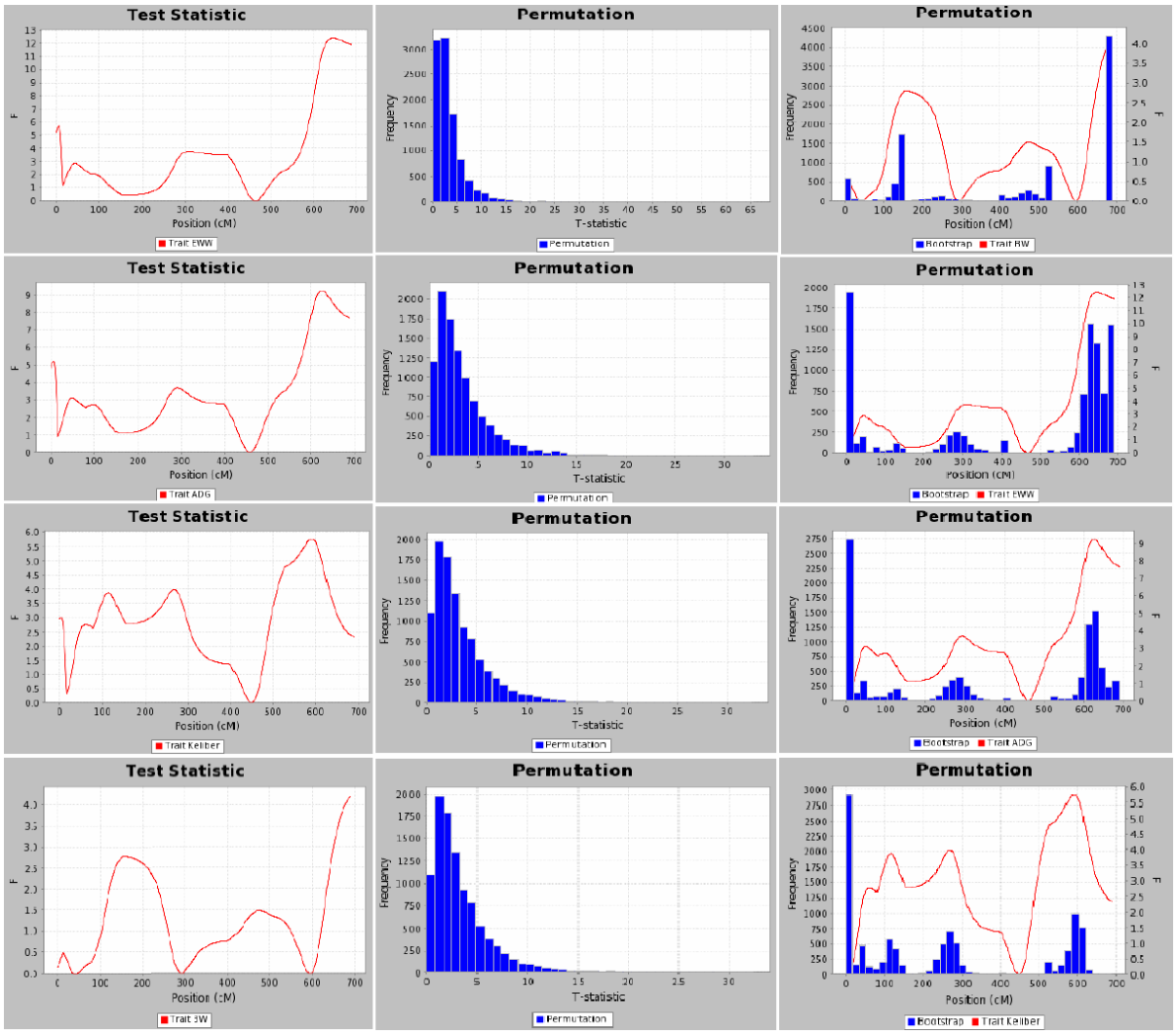

Figure 4 Permutation test results for the individual families (Half sib) and across families' analyses on 5% and 1% significant levels obtained for measured traits

Table 2 Summary of significant QTL from individual family analyses

Trait	QTL position (cM)	F-value	F-statistic (0.05)[a]	F-statistic (0.01)[b]	Sire effect	Confidence level (cM)[c]	Closest marker(s)
BW0 (kg)	689	4.2^{ns}	8.94	15.82	-0.5225±0.12	2-678	Cssm019, Cssm032
WW (kg)	654	**12.41***	**8.90**	**13.49**	-3.697±1.07	**1-689**	**Cssm019, Cssm032**
ADG (g/day)	624	**9.24***	**8.94**	**14.15**	-49.85±26.39	**0-687**	**Cssm019, Cssm032**
Keliber	591	5.75^{ns}	8.94	15.03	-1.83±0.76	0-613.5	Cssm019, Cssm032

[a] QTL location based on the sheep sex averaged linkage map (ØMaddox and Cockett, 2007).
[b] QTL effect scaled by the standard deviation of the trait.
[c] Chromosome-wide F-statistics for P < 0.05.

For BW, Stone *et al.* (1999) was reported one suggestive QTL on chromosome 1 in Brahman cattle.

POU1F1 candidate gene responsible for growth was located on OAR1 and flanking microsatellite of this region always significantly associated with growth (Woollard *et al.* 2000).

In porcine QTL mapping, also one QTL for BW was reported on chromosome 13 near POU1F1 gene (Song *et al.* 2007; Yu *et al.* 1999). In addition, identification of casual mutation for growth on POU1F1 of commercial pigs confirmed association between this gene and BW trait (Yu *et al.* 1999).

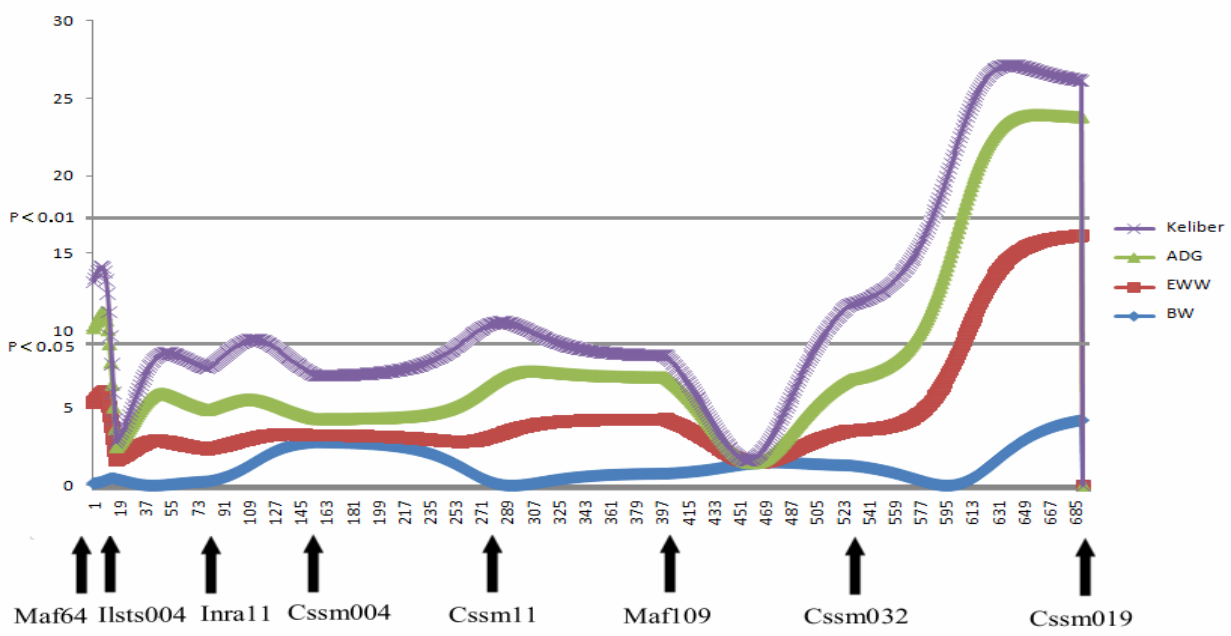

Figure 5 F-statistic curves resulted from the joint analysis of half-sib families on chromosomes 1 of sheep
The lower lines represent 5 % generated F ratio during chromosome-wide significant analysis
The upper horizontal represent 1% generated F ratio during chromosome-wide significant analysis
BW: birth weight; EWW: adjusted weaning weight; ADG: average daily gain (0-90 day) and Keliber: keliber ratio

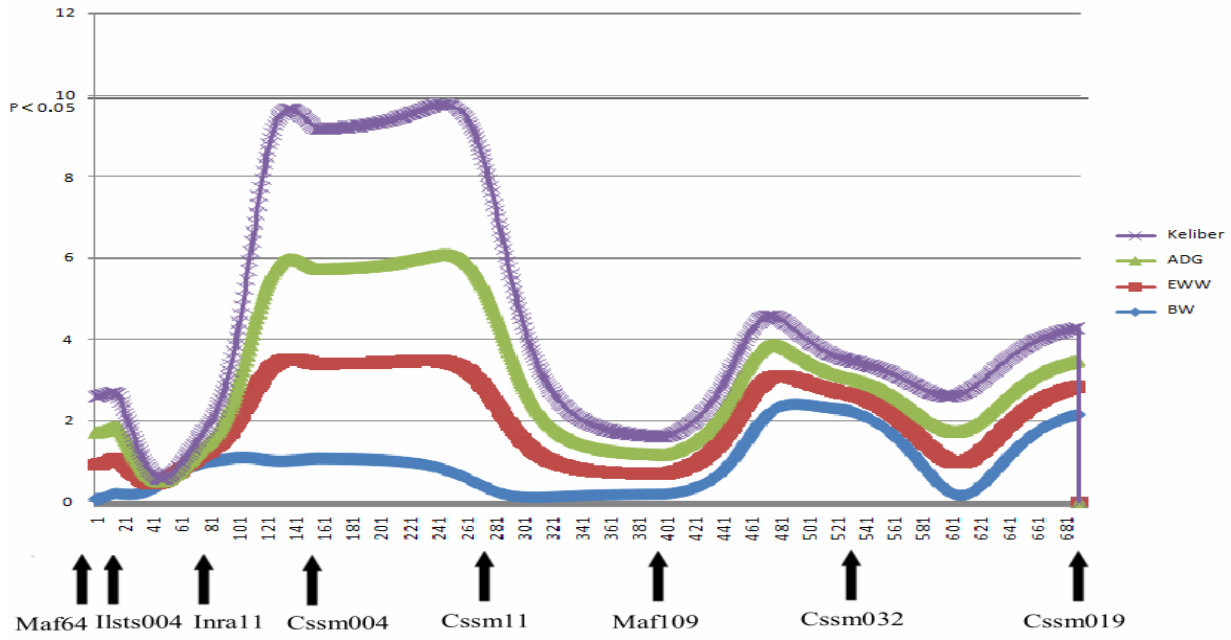

Figure 6 F-value statistics curves resulted from the joint analysis across families on chromosomes 1 of sheep
The lower lines represent 5% generated F ratio during chromosome-wide significant analysis
The upper horizontal represent 1% generated F ratio during chromosome-wide significant analysis
BW: birth weight; EWW: adjusted weaning weight; ADG: average daily gain (0-90 day) and Keliber: keliber ratio

In consistence of this claims, however, Zhao *et al.* (2004) reported there is no significant relationship between *POU1F1* gene and carcass or growth and traits in Angus beef cattle.

Evidence on existence of QTL affecting conformation traits (rump width, rump length and rump angle) in bovine chromosome 1 was emphresised by Bichard *et al.* (2003). The presence of a significant QTL on chromosome 1 of Japanese Black cattle was reported by Malau-Aduli *et al.* (2005) for chest width reinforces. Summary of related pervious literatures about QTL identification for growth in chromosome 1 in livestock (Table 3).

Table 3 Summary of related pervious literatures about QTL identification for growth in chromosome 1 in livestock

Author	Country	Breed	Marker	QTL location	QTL peak	Significant	Closer locus	Trait
Raadsma *et al.* (2009)	Australia	Awassi, Merino sheep	Microsatellite	87.3(cM)	81.03-81.23 (cM) 62.9-63.0 (Mbp)	< 0.05	BM4129	Body weight and growth
Visser *et al.* (2013)	South Africa	Angora goat	Microsatellite	-	-	none	-	Pre-weaning growth
Ravari *et al.* (2016)	Iran	Kermani sheep	Microsatellite	34(cM) 91(cM)	30-38(cM) 68-71(cM) 90-91	< 0.05 < 0.01	MAF4 DIK5034 MCM130	Growth trait
Esmailizadeh (2014)	Iran	Rayini goats	Microsatellite	103(cM)	-	< 0.01	-	Birth weight age of puberty
Asadi-Khoshoei *et al.* (2018)	Iran	Lori-Bakhtiari sheep	Microsatellite	-	210(cM) 252(cM)	< 0.05 < 0.01	INRA011 LSCV105 MCM137	Growth trait
Walling *et al.* (2004)	UK	Suffolk sheep Texel sheep	Microsatellite	BMS2321 BMS1789	227 cM	< 0.05 < 0.01	BM8246 and McM130	Growth and carcass traits
Our study	Iran	Ghezal sheep	Microsatellite		591(cM) 689(cM)	< 0.05 < 0.01	Cssm019 Cssm032	Pre-weaning growth

As logic justification, the reason for a difference in the present result with other findings seems particularly due to different sheep breed and geographical, chromosomal region, investigated microsatellite loci, genotyping technique and even more by technical staff, which may influence the output of analysis and interpretation of raw data.

It should be highlighted, however, relatively small offspring size per parental HF was affect output of QTL analysis (Göring *et al.* 2001; Esmailizadeh *et al.* 2008). Furthermore, in QTL mapping small family size imposed the wide confidence interval calculated for the QTL position.

There are limitations in this study: Natural service was routine breeding program for Ghezal sheep breeding station and number of different f half sibs families and offspring within each paternal HF families affected due this natural barrier. Only two sire exhibited heterozygotes pattern for most of investigated loci and analyses using more families and more animals will be useful to confirm or to reject these findings.

CONCLUSION

Two the QTL retained significance (P≤0.01) for BW and KRB at the region of flanking markers CSSM019-CSSM032 and BM1312 respectively. Further studies will be useful using using more families, animals, and chromosomal number for identification of co-segregation of quantitative trait loci (QTL) affecting pre-weaning traits in Ghezal fat tailed sheep.

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