RESEARCH NOTE

Associations of *POU1F1* gene polymorphisms and protein structure changes with growth traits and blood metabolites in two Iranian sheep breeds

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Introduction

The sheep *POU1F1* gene is located on chromosome 1 and contains six exons and five introns. In various mammalian species, certain mutations in different exons are associated with different production traits. The aim of this study was to investigate the single nucleotide polymorphisms (SNPs) at 3 exon of *POU1F1* gene and protein structure changes and its relationships with growth traits and blood metabolites in two Iranian sheep breeds: Zel (Z) and Lori-Bakhtiari (LB). Blood samples from 90 LB and 90 Z sheep were collected to extract the DNA. Polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP) and sequence analyses were carried out to examine the exon 3 of *POU1F1* to highlight possible SNPs. Sequence analysis showed one mutation G to A at codon 105 converting an alanine into a threonine and the three-dimensional structures predicted for *POU1F1* exon 3 were similar collectively. When *POU1F1* genotypes were tested, the animals with AA genotype had higher weaning weight than those with GG genotype ($P < 0.05$). These results imply that the *POU1F1* genotypes affect weaning weight, suggesting that this polymorphism can be used as a molecular marker for this trait.

The sheep industry is an important division among the small domestic animals sector in Iran. The sheep population in 2011 was about 54 million, including 27 breeds and ecotypes (Iranian ministry of agriculture 2011). The Iranian native breeds of sheep are multi-purpose and have significant roles in meat, milk and wool production. Meat from young or adult sheep has been consumed in Iran. So, the further improvement and increase in quantity and quality of sheep meat will better contribute to the Iranian society, particularly in economy and nutrition. This issue can be resolved by culturing more and better sheep breeds. As it is difficult to quickly culture excellent domestic breeds using the traditional breeding and genetics, currently major breeders focus on DNA markers for developing breeds through marker-assisted selection (MAS). Hence, it is important to identify significant associations of the polymorphisms within the crucial candidate genes with growth traits and blood metabolites. POU1F1 (also known as PIT-1 or GHF-1) is a tissue-specific transcription factor chiefly expressed in the anterior pituitary (Bodner *et al.* 1988; Ingraham *et al.* 1988). This protein was first associated with a certain role in the transcriptional adjustment of growth hormone (*GH*) and prolactin (*PRL*) genes (Ingraham *et al.* 1988; Nelson *et al.* 1988). It is also involved in the activation of β subunit of thyroid-stimulating hormone (TSHβ) (Li *et al.* 1990), *POU1F1* itself (Lefevre *et al.* 1987; McCormick *et al.* 1990) and growth-hormone-releasing hormone receptor (*GHRH-R*) genes (Lin *et al.* 1992). Moreover, besides its role in gene activation, POU1F1 is essential for the differentiation, reproduction and survival of somatotrope and lactotrope as well as thyrotrope cells (Li *et al.* 1990). The ovine *POU1F1* gene is located on chromosome 1 having five introns and six exons (Theill *et al.* 1992; Woollard *et al.* 2000). To date, 18 different mutations were reported diffused over the six exons, excluding exon 2. Malvagia *et al.* (2003) presented a review of 15 of these mutations, including one novel mutation detected in exon 4. Indeed, some mutations in different exons of *POU1F1* gene are associated with major functional impairment at pituitary level like the combined pituitary hormone shortage in humans (Malvagia *et al.* 2003; Salemi *et al.* 2003). In addition, *POU1F1* polymorphisms in pigs are significantly linked with birth weight, weaning weight, average daily gain and back fat thickness (Yu *et al.* 1995). Several mutations of *POU1F1* gene are in conjunction with body weight, milk proteins and fat yields in cattle (Renaville *et al.* 1997).

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Recently, several polymorphisms were reported in goat *POU1F1* gene (Lan *et al.* 2007a, c) which were related to milk yield, litter size and body weight (Lan *et al.* 2007b, c). Further, in ovine species several polymorphisms have been identified in the recent years which showed no relationship with milk traits (Mura *et al.* 2012). There are few studies on *POU1F1* in sheep breeds (Bastos *et al.* 2006; Mura *et al.* 2012) but no publications are available to evaluate the association of this marker with growth traits and blood metabolites in sheep. The objectives of this study were to identify *POU1F1* gene mutations by PCR-SSCP and DNA sequencing methods and evaluation of the association between these mutations and protein structure changes with growth traits and blood metabolites in two Iranian sheep breeds.

Material and methods

Animals

This study has been conducted in two different Iranian sheep breeds: LB (fat-tail) and Z (thin-tail). Ninety LB sheep from Shahrekord's research station and 90 Z sheep from Gorgan's research stations were used. The two sheep breeds had no genetic assosiation history. These two breeds differed in size and living conditions. The sheep population located in the western part of Iran is predominatly related to LB breed, a fat-tailed dual purpose sheep, large in size and white in colour. The Z sheep is a native sheep in the two northern provinces of the country. This breed is small in size, early maturing and mostly brown. Blood samples were collected from each animal to measure triglyceride (TR) and cholesterol (CL). Data of birth weight (BW), weaning weight (WW), body length (BL), body height (BH), heart girth (HG), thigh girth (TG) and abdominal girth (AG) were measured and recorded. All 180 animals were used for genomic analysis.

DNA extraction and amplification

Genomic DNA was extracted from whole blood using salting out method (Miller *et al.* 1988) and preserved at −20°C until usage. In order to amplify 295 bp of *POU1F1* gene, 100 ng of genomic DNA including the partial coding regions of the *POU1F1* gene were used with the primer reported by Bastos *et al.* (2006). The PCR was conducted in a 25 μL reaction mixture containing 100 ng of DNA, 2 mM of $MgCl_2$, 0.2 mM of dNTPs, 12 pmol of each primer and 1 U of *Taq* DNA polymerase. PCR conditions were as follows: denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for 4 min. Electrophoresis of PCR products were performed in 1% (w/v) agarose gel in parallel with 100 bp DNA marker, in 1× TAE buffer at a fixed voltage of 90 V for 30 min. After ethidium bromide colouration, products were visualized by ultraviolet transillumination.

SSCP analysis

All PCR products were subjected to SSCP analysis. Aliquots of 4 μL PCR products were mixed with 12 μL denaturing solution (A: 95% formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue; B: same as A, plus 20 mM of EDTA), denatured by heating at 96°C for 10 min and immediately placed on wet ice. Denatured samples were loaded on 12% PAGE gel in 0.5× TBE buffer at a constant voltage of 300 V for 15 h. The gel was stained by a silver staining method (Sanguinetti and Simpson 1994). The PCR fragments from different SSCP patterns were sequenced in both directions.

Protein structure

We studied the structure and changes in the POU1F1 protein using the methods of Bahrami *et al.* (2012). Using the MEGA4 software (Tamura *et al.* 2007), we managed to draw the phylogenetic tree of the relevant proteins.

Statistical analysis

Genotypic, allelic frequencies and Hardy–Weinberg equilibrium (HWE) were estimated with the use of GenAlEx 6.41 software (Peakall and Smouse 2006). Associations of the animal genotypes with growth traits and blood metabolites were calculated by analysing variance of quantitative traits for all the traits mentioned, using the general linear model of SAS (2004). The equation models for the analysis were:

(i) $Y_{ijklm} = \mu + G_i + B_j + S_k + T_l + M_m + b_1(D_{ijklm} - D' + e_{ijklm})$,

(ii) $Y_{ijklm} = \mu + G_i + B_j + S_k + T_l + M_m + b_1(D_{ijklm} - D' + b_2(AW_{ijklm} - AW') + b_3(BW_{ijklm} - BW') + e_{ijklm})$,

(iii) $Y_{ijklm} = \mu + G_i + B_j + S_k + T_l + M_m + b_1(D_{ijklm} - D' + b_4(A_{ijklm} - A' + e_{ijklm})$.

The eqs (i), (ii) and (iii) were used for the analysis of birth weight trait, weaning weight trait and other traits (TR, CL, BL, BH, BC, TC and AC), respectively. In these models, Y_{ijklm} was the phenotypic value of the trait; μ the population mean; G_i the fixed effect of the ith genotype; B_j the fixed effect due to the jth breed; S_k the fixed effect due to the kth sex; T_l the fixed effect due to the lth type of birth; M_m the fixed effect due to the mth month of birth; b_1 regression coefficient of y on D; D_{ijklm} the age of dam (in days); b_2 regression coefficient of y on AW; AW_{ijklm} the age of weaning weight (in days); b_3 regression coefficient of y on BW; BW_{ijklm} the birth weight, b_4 regression coefficient of y on A; A_{ijklm} the age of animal (in days) and e_{ijk} represent the random residual effects.

Results

SSCP analysis

The PCR-SSCP analysis of *POU1F1* exon 3 revealed three distinct patterns (figure 1). There were three genotypes

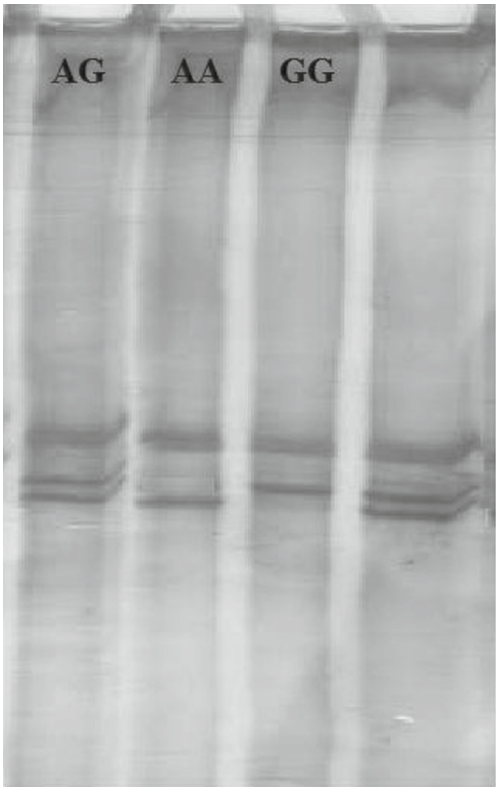

Figure 1. PCR-SSCP genotypes of complete exon 3 of the *POU1F1* gene in LB and Z sheep.

namely, GG, AG and AA in this piece. The genotypic frequencies are shown in table 1 for the two breeds. Two different alleles A and G were identified (figure 1). All alleles were present in the two studied breeds, but in different proportions. There was a high genetic diversity within ovine *POU1F1* gene in the analysed populations (table 1). In LB sheep, breed A allele and in Z breed G allele were more frequent. In table 1 HWE ($\chi 2$) test showed that the population of LB sheep is in HWE but not in the population of Z breed ($P < 0.01$). From the results, it appears that PCR-SSCP is a potential method for identifying the genetic variants. The benefit of using SSCP technique is that by neutral polyacrylamide gel electrophoresis (PAGE), two single-stranded DNA fragments in which the nucleotide sequences differ at only one position in fragments of genomic DNA (Orita *et al.* 1989).

Table 1. Allele and genotype frequencies of the *POU1F1* exon 3 and the test Hardy–Weinberg for level of significance of the deviation within breeds.

	Genotype			Allele		
Breed	GG	GA	AA	G	A	HWE
LB	0.27	0.40	0.33	0.47	0.53	0.105
Z	0.51	0.23	0.26	0.63	0.37	0.0001

LB, Lori-Bakhtiari; Z, Zel.

DNA sequence analysis and protein structure changes of POU1F1 gene exon 3

Sequences of PCR amplicons from a representative of the unique PCR-SSCP variants were analysed and compared to NCBI reference sequence AJ549206.1 (Bastos *et al.* 2006). Sequence analysis showed that there was one mutation G to A at codon 105 converting an alanine into a threonine (figure 2). This mutation was previously reported by Bastos *et al.* (2006). This polymorphism is located in the region between the transactivation domain and the POUs domain, named CHG, which is rich in charged amino acids. Further, the three-dimensional structures predicted for *POU1F1* exon 3 were similar together (figure 3). The *POU1F1* phylogeny tree revealed that the ovine *POU1F1* mRNA sequence showed high similarity with cow and low similarity with that of turkey and poultry (figure 4).

Association of polymorphisms with growth traits and blood metabolites in sheep

The results on the correlation between genotypes at exon 3 of *POU1F1* gene with phenotypic data of growth traits and blood metabolites are shown in table 2. The animals with AA genotype have higher weaning weight than those with GG genotype ($P < 0.05$) (table 2). However, there was no significant difference between genotypes for other traits studied in these populations ($P > 0.05$).

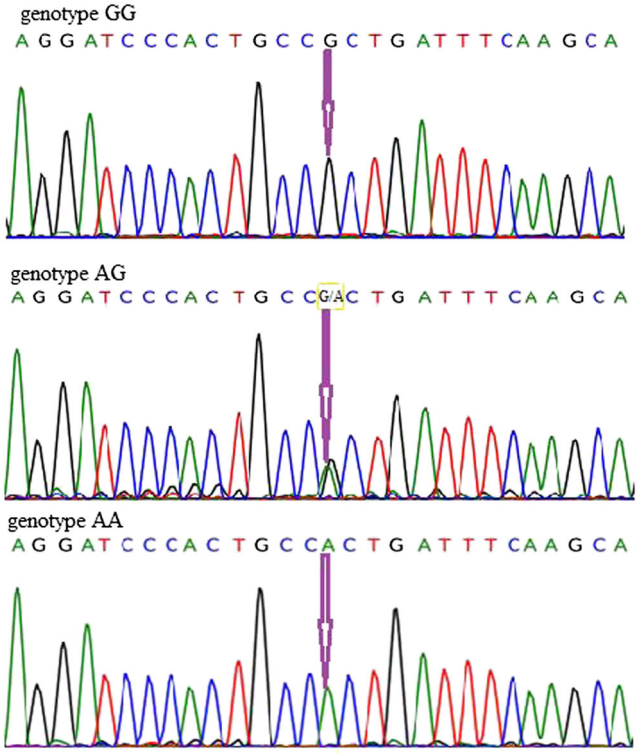

Figure 2. The sequencing of genotypes namely, GG, AG and AA in exon 3 of the *POU1F1* gene in LB and Z sheep.

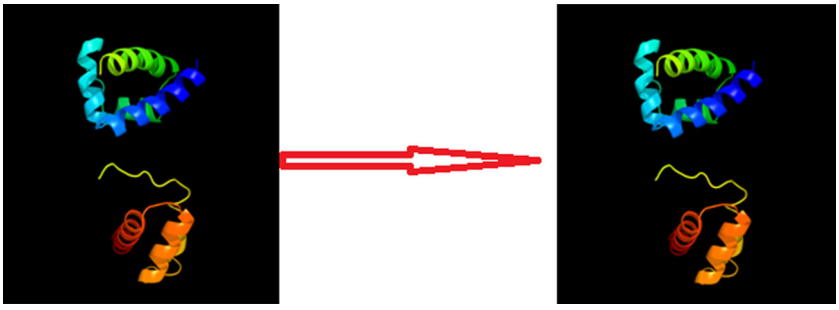

Figure 3. The change of amino acids and resulting changes in POU1F1 protein structure of LB and Z sheep (the change of alanine to threonine at the codon 105).

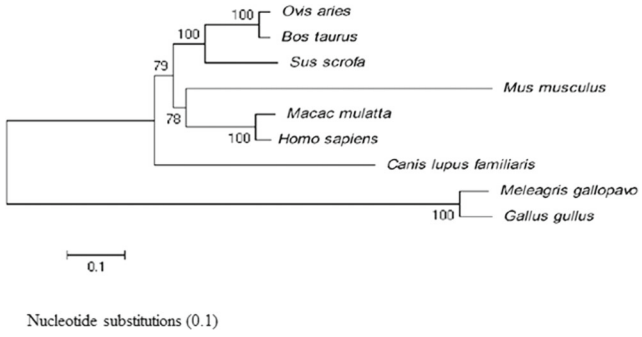

Figure 4. Phylogeny tree of *POU1F1* gene sequences drawn using the ClustalW and MEGA4 methods of sequence alignment.

Table 2. Association of the *POU1F1* exon 3 genotypes with growth traits and blood metabolites (mean ± S.E.).

Traits	Genotype		
	GG	AG	AA
BW (kg)	4.63 ± 0.18	4.5 ± 0.17	4.51 ± 0.17
WW (kg)	24.4^{a} ± 0.79	25.1^{ab} ± 0.83	26.9^{b} ± 0.83
TR (mg/dL)	31.82 ± 5.62	32.64 ± 4.6	34.48 ± 4.9
CL (mg/dL)	51.19 ± 7.75	49.22 ± 6.28	53.56 ± 6.83
TG (cm)	30.24 ± 1.05	31.56 ± 0.94	30.53 ± 0.92
BH (cm)	66.52 ± 1.4	68.14 ± 1.25	67.95 ± 1.22
BL (cm)	61.42 ± 1.28	61.48 ± 1.14	61.07 ± 1.12
HG (cm)	90.23 ± 2.31	89.5 ± 2.06	90.06 ± 2.02
AG (cm)	95.17 ± 2.44	95.18 ± 2.18	95.59 ± 2.13

BW, birth weight; WW, weaning weight; TR, triglycerides; CL, cholesterol; TG, thigh girth; BH, body height; BL, body length; HG, heart girth; AG, abdominal girth.
[a,b]Values with different superscripts within the same row differ significantly ($P < 0.05$).

Discussion

Sequencing exon 3 of *POU1F1* showed the same nucleotide sequence of the corresponding region deposited in GenBank (AJ549206) and the polymorphism detected in this study is in agreement with Bastos *et al.* (2006). These authors found two SNPs in exon 3 *POU1F1*, a G to A transition altering a glycine to an asparagine at codon 89 and another G to A transition at codon 105 converting an alanine into a threonine in Churra da Terra Quente sheep breed. It was reported that there was positive association between *POU1F1* gene polymorphisms with growth and carcass traits in pigs (Yu *et al.* 1995). Further, the *POU1F1* gene regulated expression of *GH*, *PRL*, *TSHβ* gene and *POU1F1* itself (Sun *et al.* 2002). In addition, *POU1F1* gene is considered to affect performance traits which would be profitable for the sheep industry, whose DNA markers will help in animal selection and breeding via marker-assisted selection. This study is the first to investigate the role of *POU1F1* in growth traits and blood metabolites in sheep. With this polymorphism and the genetic diversity observed between the breeds, we considered to highlight whether there were significant associations between the *POU1F1* gene polymorphism and growth traits and blood metabolites in the sheep breeds. When *POU1F1* genotypes were tested, animals carrying AA genotype showed higher weaning weight than those with GG genotype. The previous studies on *POU1F1* gene polymorphism did not consider the associations between genotypes and growth traits and blood metabolites in sheep. It has been shown that there was an association between polymorphisms of intron 5 and exon 6 in relation to breast circumference and body length in Nanyang cattle. Xue *et al.* (2006) and Zhao *et al.* (2004) reported that there was no association between polymorphisms at intron 3 related to birth weight, weaning weight and average daily gain, in Angus beef cattle. Further, polymorphisms of *POU1F1* gene were found to be associated with milk yield and growth traits in goat (Lan *et al.* 2007c; Lan *et al.* 2009). In addition, several mutations of *POU1F1* gene are in conjunction with body weight, milk proteins and fat yields in cattle (Renaville *et al.* 1997). The above results imply that the *POU1F1* genotypes affect weaning weight, suggesting that this polymorphism can be used as a molecular marker for this trait.

Conclusion

In conclusion, it can be assumed that exon 3 *POU1F1* in these Iranian indigenous sheep breeds has high variability and the SNP observed in this study are associated with weaning weight trait. The mapping and linkage characterization

of sheep *POU1F1* gene needs to be studied in more detail, and the exact mechanism of *POU1F1* gene polymorphism contributing to growth also requires further investigation.

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