A PCR-RFLP investigation on *PROP1* gene polymorphism and its association with milk production and growth traits in Mahabadi goats

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Abstract Prophet of POU1F1 (*PROP1*) plays direct or indirect important roles in the morphogenesis of pituitary gonadotropes as well as lactotropes, somatotropes and caudomedial thyrotropes. It also controls the expression of growth hormone, prolactin and thyrotropin subunits through regulatory PIT1 factor. These hormones have important influences on production traits in most animal species including the goat. Therefore, *PROP1* gene could be considered as a candidate gene for milk production. The objective of this study was to determine the polymorphism of *PROP1* gene and its association with milk yield and growth traits in Mahabadi goats. We investigated the mutation (GenBank accession number: AY533708.1) within exon 2 of *PROP1* gene by using the *Hin*6I PCR-RFLP method.This polymorphism alters the amino acid at codon 79 from alanine to valine in *PROP1* protein. Only two genotypes CC and CT (frequencies 0.9 and 0.1 respectively) were observed. Polymorphism of *PROP1-Hin*6I locus showed significant relationship with milk production ($P < 0.01$), milk fat percentage ($P < 0.05$), milk protein percentage ($P < 0.05$), somatic cell count ($P < 0.05$) and daily weight gain ($P < 0.05$). Therefore, *PROP1-Hin*6I locus could be considered as a molecular marker for milk production and milk composition traits in Mahabadi goats.

Keywords: Mahabadi goat, association analysis, PROP1 gene, production traits

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Introduction

Milk production and composition are important production traits in dairy animals including the goats (Li *et al.*, 2012). Likewise, growth is considered as a main economic trait in the goat industry. Therefore, finding and validating the genetic markers related to growth and milk traits are essential in establishing a marker assisted selection (MAS) system (Hua *et al*., 2009).

PROP1 is expressed in the pituitary gland, and plays an important role in the morphogenesis of the pituitary gonadotropes as well as lactotropes, somatotropes and caudomedial thyrotropes. It also controls the expression of growth hormone (GH), prolactin (PRL) and thyroid stimolating hormone (TSH) subunits, through regulatory PIT1 factor (Xu *et al*., 2010). It has been demonstrated that *PROP1* mutations are responsible for deficiencies in PRL, GH and TSH (Wu *et al*., 1998).

Two basic regions (B1 and B2) have been identified within the homeo domain of the PROP1 protein, which are important for nuclear localization, target gene activation and DNA binding. These regions are close to amino acids 69 - 73 and 120 – 126 of the protein molecule, respectively (Guy *et al.*, 2004). Some of the missense mutations in the *PROP1* gene in the human, causing amino acid changes in B1 and B2 regions, are associated with combined pituitary hormone deficiency (CPHD) diseases (Guy *et al.*, 2004; Cushman *et al.*, 2002). This has an autosomal recessive mode of inheritance and is characterised by GH, TSH, PRL, LH, FSH and ACTH deficiencies (Wu *et al.*, 1998). Mice *PROP1* mutations have been found to be associated with Ames dwarfism (Sornson *et al.*, 1996; Nasonkin *et al.*, 2004).

In addition, *PROP1* is adjacent to the AT-rich region (80%) in about -800 bp up-stream of the porcine FSHβ gene, resulting in the transcription of the FSHβ gene (Aikawa *et al*., 2005). The *PROP1* gene organizes 3 exons encoding for the 226 amino acids in cattle, sheep and goats (Carvalho *et al.*, 2006; Sloop *et al.*, 2000). This gene is located on chromosome 5 (Savage *et al.*, 2003).

Considering *PROP1* gene of different domestic animals, eleven SNPs in ovine (Zeng *et al*., 2011), five mutations of *PROP1* gene in bovine (Pan *et al*., 2007) and four SNPs in porcine (Xiaohui and Yuhong, 2007) have been reported. Only one mutation in all (three) exons of *PROP1*

gene of goat were found and its association with production traits in cashmere and dairy goat breeds (Lan *et al*., 2009b), and with growth traits in one of the meat goat breeds (Xu *et al*., 2010) has been investigated. Positive associations with body length and chest width were reported (Xu *et al*., 2010). On the other hand, associations with body weight, cashmere yield, fiber length, wool thickness and milk yield traits were not significant (Lan *et al*., 2009b).

The presence and the effect of such mutations, if any on growth and milk production traits in Mahabadi goats have not been reported. Mahabadi goat is a dual purpose milk and meat breed and is a native of western Iran. The purpose of present study was to investigate the polymorphism of *PROP1* gene and its association with growth and milk traits in Mahabadi goat.

Materials and methods

DNA samples

Blood samples (5 ml of each) were collected from the jugular vein of 140 Mahabadi goats and kids (100 does and 40 male kids). Genomic DNA was extracted using salting-out method (Miller *et al*., 1988). The quality of the DNA was checked on 1% agarose gel and the quantity was measured by UV spectrophotometry at A260/A280 nm.

DNA amplification

Based on the sheep *PROP1* gene sequence (GenBank accession No. AY533708), the primers were designed using the VectorNTI software[1]. The sequence of the primers were as follows:

Forward primer:

5'-GATGGATGGATGGGTCTCTG -3 '

Reverse primer:

5'-TGGTGAAGGTTTGGGTTAGG -3'

Genotyping for *PROP1* polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A single reaction medium (25 µl) contained 50 to 100 ng of genomic DNA, 10 pmol of each primer, 0.2mM dNTP, 1.5 mM $MgCl_2$, 1x PCR buffer and 1 Unit of Taq polymerase (Genet Bio, Korea) .The cycling protocol was 5 min of 94°C, 35 cycles of 94°C for 1 min, annealing at 58°C for 1 min, 72°C for 1 min with a final extension step at 72°C for 5 min.

Hin6I PCR-RFLP analysis

The PCR product was digested with *Hin6*I enzyme (Fermentase, Germany) in a total volume of 15 µl (1.5 µl buffer Tango, 7 U of *Hin6*I, 2.8 µl distilled water and 10 µl PCR product) and was incubated at 37 °C overnight. The fragments were separated on a 3% agarose gel by electrophoresis (77 W; 90 min). Agarose gels were stained with "DNA Safe stain" and viewed under the UV light.

Statistical analysis

Genotypic frequencies, allelic frequencies, and Hardy–Weinberg equilibria were estimated using the GenAlEx 6.41 software (Peakall and Smouse, 2006). Analysis of polymorphic *PROP1* gene was performed using the SAS software (SAS Institute, 2004). Traits analyzed in the present study included the milk yield, milk fat percentage, protein percentage, somatic cell count (SCC), birth weight, weaning weight (adjusted to 115th day of

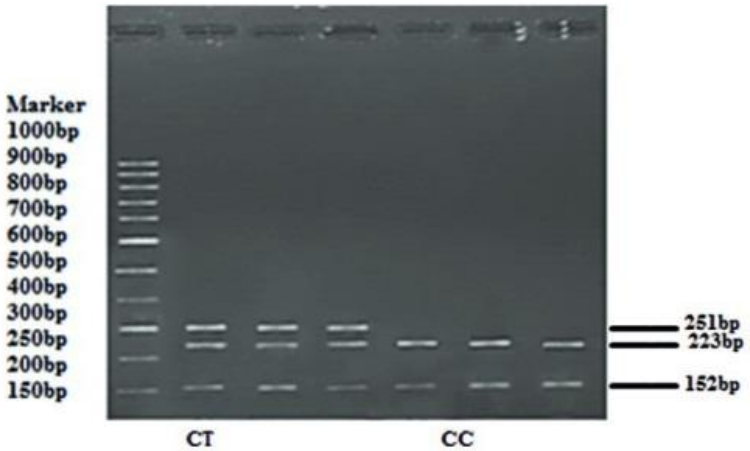

Fig 1. Gel electrophoresis of PCR product after digestion with *Hin6*I restriction enzyme.(Marker Fermentas, Germany).

[1]Vector NTI is a bioinformatics software package, Molecular biology Suite of Sequence Analysis & Design Software Tools.

Table 1. Descriptive statistics of phenotypic traits in Mahabadi goats

Traits	N	Min.	Max.	Mean	STD	CV
Milk yield (g) [1]	855	100	2200	633	353.93	55.91
Fat (%)[1]	204	2.00	10.06	2.28	1.81	79.45
Protein (%)[1]	204	2.29	6.87	4.15	0.65	15.65
SCC×1000 (per ml) [1]	202	7	13853	963	1990	206.54
Birth weight (kg)	40	2.18	4.60	3.26	0.61	18.76
Weaning weight (kg)	40	9.20	30.50	19.62	5.05	25.74
Daily weight gain (kg)	40	0.06	0.33	0.14	0.05	37.80
Final weight (kg)	40	18.60	42.83	29.55	6.06	20.51

[1] Test-day

age), daily weight gain from birth to weaning, and slaughter weight after an 80-day feedlot period. The Shapiro-Wilk test was used for testing the normality of data. Box-Cox transformation was applied to the data that were not normaly distributed, using the PROC TRANSREG of SAS software. Milk production traits and growth traits were analyzed using the Proc Mixed and Proc GLM.

The following model was used to analyze the association of different genotypes with milk production traits:

$y_{ijk=} \mu + G_i + M_j + b\,(W_{ijk} - \overline{W}) + E_{ijk}$

where y_{ijk} is the observation on trait (milk yield, fat percentage, protein percentage, SCC) measured in the k^{th} animal, μ is the population mean, G_i is the fixed effect of the i^{th} PROP1 genotype (CC, CT; i=1, 2), M_j is the fixed effect of the j^{th} recording month (March, April, May, June, j = 1, 2, 3, 4), b $(W_{ijk} - \overline{W})$ is the regression of y on animal body weight at kidding and E_{ijk} is the random residual effects.

The following model was used to analyze the relationship between different genotypes and growth traits:

$y_{ijkl} = \mu + G_i + M_j + Y_k + b_1\,(W_{ijkl} - \overline{W}) + b_2\,(D_{ijkl} - \overline{D}) + E_{ijkl}$

where y_{ijkl} is the observation on trait (birth weight, weaning weight, daily weight gain, final weight) measured in the l^{th} animal, μ and G_i are the same as in the previous model, M_j is the fixed effect of the j^{th} birth month (April, November, January, February, March; j = 1, 2, 3, 4, 5), Y_k is the fixed effect of the k^{th} birth year (2010-2011, 2011-2012; k=1, 2), b_1 $(W_{ijkl} - \overline{W})$ is the regression of y on body weight at recording time, b_2 $(D_{ijkl} - \overline{D})$ is the regression of y on the number of days from birth to weaning and E_{ijkl} is the random residual effects.

Results and Discussion

Genotyping at the PROP1- Hin6I locus

In PCR-RFLP analysis, the 403 bp fragment of PROP1 gene was digested with *Hin6*I endonuclease enzyme. The genotype CC had three fragments (223, 152 and 28 bp), the genotype TT had two fragments (251 and 152 bp) and the genotype CT had four fragments (251, 223, 152 and 28 bp) (Figure 1).Two genotypes (CC and CT) were identified in the studied population of Mahabadi goats. Polymorphism in *PROP1-Hin*6I locus (g. 1795C>T) alters the amino acid at codon 79 from alanine to valine.

Genotypic and allelic frequencies of the PROP1-Hin6I locus

The frequencies of genotypes CC and CT were 0.9 and 0.1, and those of C and T alleles were 0.95 and 0.05, respectively .

Genotypes were distributed according to the Hardy-We-

Table 2. The effect of genotypes of PROP1- *Hin6I* locus on milk production traits in Mahabadi goats (LSMeans ± SE)

Traits	CT Genotype	CC Genotype	*P* value
Milk yield (g)	27.09±3.76	21.53±3.5	0.001**
Milk fat (%)	0.09±0.3	0.45±0.25	0.049*
Milk protein (%)	0.36±0.001	0.37±0.0008	0.010*
SCC×1000 (per ml)	5.00±0.48	4.02±0.26	0.034*

* $P < 0.05$

** $P < 0.01$

inberg equilibrium (P > 0.05). For this locus, the number of alleles (Na) was 2, the number of effective alleles (Ne) was 1.1, the observed heterozygosity (Ho) was 0.1 and the expected heterozygosity (He) was 0.095. Shannon's Information Index (I) was 0.2, indicating a low genetic diversity in *PROP1* gene in this sample of Mahabadi goat population.

Descriptive statistics of phenotypic records in Mahabadi goats

Descriptive statistics (number of records, minimum, maximum, mean, standard deviation and coefficient of variation (CV)) of the raw data related to milk and weight records, are presented in Table 1 .

The CV was high for SCC trait, reflecting inconsistencies among the samples within the group, the high CV and trait variability can be related to the method of measuring the trait.

Association of elements of model (except genotypes) with milk production and growth traits

The doe weight at kidding affected the milk protein percentage (P<0.05), and the recording month had a significant effect on milk yield (P<0.01) and SCC (P <0.05).

The number of days from birth to weaning significantly affected the weaning weight (P<0.05), birth year affected the weaning weight (P<0.05) and daily weight gain (P<0.01), doe weight at kidding affected the birth weight (P<0.01), weaning weight affected the final weight (P<0.01), and month of birth affected the weaning weight (P<0.01) and daily weight gain (P<0.01).

Association of PROP1-Hin6I polymorphism with milk production traits

Relationships between genotypes (TT and TC) and milk production traits was determined in 100 Mahabadi does. Least squares means and standard errors of milk production traits (after normalization of the data) for PROP1 gene are shown in Table 2.

The results indicated that g.1795C>T SNP was significantly associated with higher milk yield (P<0.01), lower milk fat percentage (P<0.05), lower milk protein percentage (P<0.05) and more SCC (P<0.05). Goats with CT genotype produced more milk yield than did the CC genotype. Milk produced by CC genotype contained higher fat and protein percentages, but fewer SCC number, compared with the CT genotype. Previous studies in goats, reported no significant relationship between polymorphism in *PROP1-Hin6*I locus and milk yield (Xu *et al*., 2010). Therefoe, the *PROP1* gene in goats might be considered as a candidate gene for milk production traits (milk yield, milk fat%, milk protein% and SCC).

Association of PROP1-Hin6I polymorphism with growth traits

The relationships between genotypes (CC and CT) and growth traits was studied using the data on 40 Mahabadi kids. The least squares means and standard errors of growth traits (after normalization of the data) are shown in Table 3.

Association analysis results showed that polymorphism in *PROP1-Hin6*I locus did not affect the birth weight, weaning weight and final weight (P>0.05), but had a significant effect on daily weight gain (P<0.05). The goats with the CT genotype recorded lower daily weight gain than did the CC genotype. Other studies in goats reported no significant relationship between polymorphism in *PROP1*-Hin6I locus and body weight (Lan *et al*., 2009b; Xu *et al*., 2010). However, based on the results of this study CC genotype might be used as a molecular marker for higher daily weight gain in Mahabadi goat.

The *PROP1* mutations induce a similar phenotype considering GH, prolactin (PRL) and thyroid stimulating hormone (TSH; Mendonca *et al*., 1999; Paracchini *et al*., 2003). It is known that PRL, GH and TSH genes are significantly associated with reproduction, growth and development and also fiber traits in mammals (Esperante *et al*., 2008; Gupta *et al*., 2007; Lan *et al*., 2009a); therefore, the goat *PROP1* gene might be considered as a candidate gene for selection for production traits through MAS. Previous studies in goat also showed that

Table 3. The effect of genotypes of PROP1- *Hin6I* locus on growth traits in Mahabadi goats (LSMeans ± SE)

Traits	CT Geotype	CC Geotype	*P* value
Birth weight (kg)	3.29±0.34	3.06±0.18	0.485^{ns}
Weaning weight (kg)	21.78±2.42	17.03±1.8	0.1082^{ns}
Daily weight gain (kg)	0.15±0.008	0.17±0.005	0.0419^{*}
Final weight (kg)	29.67±0.96	29.37±0.72	0.794^{ns}

* P < 0.01

ns: non-significant.

PROP1 gene polymorphisms were significantly associated with body size (Xu *et al*., 2010). In the present study we also recorded significant statistical differences in milk production traits and daily weight gain between different genotypes.

Conclusions

The results of analysis revealed the association of polymorphism of *PROP1-Hin6*I at SNP g.1795C>T in milk production ($P<0.01$), milk fat percentage ($P<0.05$), milk protein percentage ($P< 0.05$), SCC ($P<0.05$) and daily weight gain ($P<0.05$). However, in this study no significant association of different genotypes with other growth traits of goat was detected. Based on the findings in this research it might be suggested that CT genotype could be considered as a molecular marker for milk yield, and CC genotype could be regarded as a molecular marker for more milk fat percentage, more milk protein percentage, less SCC and more daily weight gain in Mahabadi goat**.**

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مطالعه چندشکلی ژن PROP1 و ارتباط آن با صفات رشد و شیر در بز مهابادی

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چکیده پروتئین عامل تنظیمی PROP1 در سازماندهی بعد از تولد در گسترش غده هیپوفیز، تا رسیدن آن به بلوغ و به‌طور مستقیم و غیر مستقیم در رشد، تکامل و تنظیم فعالیت سلول‌های هیپوفیز پیشین شامل سلول‌های تولید کننده هورمون رشد، پرولاکتین و محرک تیروئید، نقش دارد. این عامل تنظیم کننده هورمون های پیش گفته است که این هورمون ها روی صفات تولیدی و رشد موثر هستند. بنابراین ژن PROP1 می تواند به عنوان یک ژن کاندیدا برای صفات رشد و تولید شیر در دام ها باشد. هدف از این مطالعه بررسی چندشکلی ژن PROP1 و شناخت ارتباط آن با صفات رشد و تولید شیر در بزهای نژاد مهابادی می‌باشد. به همین منظور قطعه مرتبط با یک جهش در درون اگزون دو ژن PROP1 بوسیله روش PCR-RFLP با کمک آنزیم برشی *Hin*6I ژنوتیپ شد. این جهش باعث تبدیل آلانین به والین در اسیدآمینه ۷۹ پروتئین PROP1 بز می‌شود. دو ژنوتیپ (CC و CT) در ژن مورد مطالعه مشاهده شد که فراوانی ژنوتیپی آن‌ها به ترتیب ۰/۹ و ۰/۱ بود. جهش جایگاه PROP1-*Hin6I* (سیتوزین به تیمین) ارتباط معنی‌داری با تولید شیر($P<0.01$)، درصد چربی شیر($P<0.05$)، درصد پروتئین شیر($P<0.05$)، تعداد سلول‌های بدنی($P<0.05$) و افزایش وزن روزانه($P<0.05$) داشت. بنابراین جایگاه PROP1-*Hin6I* می‌تواند به‌عنوان نشانگر مولکولی برای رشد تولید شیر در بز مهابادی محسوب شود.