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Polymorphisms at Exon 4 of the Prolactin Gene in Najdi and Naeimi Sheep of Saudi Arabia

Ahmed Hossam Mahmoud

Zoology Department, College of Science, King Saud University,
P.O. Box 2455, Riyadh11451, Saudi Arabia.

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Prolactin (PRL) hormone plays an important role in many biological processes in animals and humans, such as mammogenesis, lactogenesis and galactopoiesis. PRL is a single-chain protein of 199 amino acids (AA) in human, sheep, pigs and cows. The objective of the present study was to determine the polymorphism in exon 4 of *PRL* gene in 41 Najdi and 36 Naeimi sheep breeds of Saudi Arabia. A fragment of 156 bp of exon 4 of the Prolactin gene was amplified and sequenced. Our mutations were detected at positions (05, 109 and 127) of exon 4. In addition, the three mutations were used to construct different haplotypes, four haplotypes were recovered, H1 (CGG), H2 (CGT), H3 (AGG) and H4 (CCG). The H1 (CGG) was the most common haplotype and found in Najdi and Naeimi individuals with frequencies of 0.927% and 1.00% for Najdi and Naeimi sheep breeds, respectively. The haplotypes H2, H3 and H4 were unique for Najdi sheep with frequency of 0.025% for each. Naeimi sheep breed was monomorphic at exon 4 of the Prolactin gene. The present study provides basic information to understand the genetic Characterization of local sheep breeds in Saudi Arabia.

Key words: Prolactin gene, Najdi and Naeimi sheep, Single Nucleotide Polymorphism.

Genetic variability assessment of the Saudi Arabian indigenous sheep breeds is important to preserve genetic resources and to develop breeding programs to enhance production. As sheep meat production is the main source of income in local commercial flocks, increasing growth and feed utilization have always been an important breeding goal. The population of sheep in Saudi Arabia is exceeding 11.5 million head (FAOSTAT, 2013). The Molecular genetic techniques for genetic improvement are currently available for direct genotyping individuals for specific genetic loci (Agarwal *et al*., 2008).Single nucleotide polymorphisms (SNPs), one base changes including deletion, insertion and substitution, may play important roles in the regulation of genes transcription and amino acids sequences of mature proteins, which has been used to the association studies between candidate genes and complex traits in domestic animals (Kim *et al*., 2005; Meng *et al*., 2005; Yoon *et al*., 2005). The prolactin gene (*PRL*) encodes an essential hormone for initiation and maintenance of lactation, milk protein genes expression, osmoregulation, growth and development, reproduction and immune functions (Zukiewicz *et al*., 2012). It is necessary for the initiation and maintenance of lactation; it acts at the level of mammary alveoli, promoting synthesis and secretion of proteins, lactose, lipids, and other important components of milk (Leprovost *et al*., 1994; Dai *et al*., 2007). It also regulates immunological functions and participates in cell differentiation and growth (Loretz and Bern, 1982). Moreover, it is an immunomodulating molecule with relevant physiological effects, being considered as a cytosine. The PRL molecule can be linked to

* To whom all correspondence should be addressed.
E-mail:

different groups: it can be glycolized, dimerized, polymerized or hydrolyzed to originate different variants (Méndez *et al*., 2005). PRL secretion does not differ between high and low milk production. However, some researchers have found that it increases its metabolism and distribution between days 30 and 150 of lactation (Collier *et al*., 1984). Significant associations were detected between PRL polymorphisms and reproduction (Dai *et al*.,2007).While polymorphism in the *PRL* gene has been investigated in exogenous ovine genomes, there is not a single study that investigates *PRL* gene allelic variation in Saudi indigenous sheep population. The Najdi and Naeimi sheep are fat-tailed, adaptable to the prevailing adverse environment and are considered the breeds of choice among Saudi consumers (Abouheif *et al*., 1989). Therefore, the objective of this study was to characterize the polymorphism in PRL exon 4 gene in Najdi and Naeimi sheep of Saudi Arabia.

MATERIALSAND METHODS

Samples collection and DNA extraction

A total of 77 individual sheep were selected from eleven flocks representing two breeds in Saudi Arabia, namely Najdi (n = 41), Naeimi (n = 36). Ten mL of blood samples were collected from each individual sheep by jugular venipuncture into vacuum EDTA tubes. Genomic DNA was extracted using the QIA gen DNeasy blood and tissue DNA extraction kit (Hilden, Germany) following the manufacturer's instructions. The quantity and quality of DNA were checked by Jenway Genova spectrophotometer (Krackler Scientific Incorporation, USA). The O.D. 260/280 ratios were between 1.7 and 1.9 indicating high quality DNA as indicated by (Sambrook *et al*., 1989).

SNPs Identification and Genotyping

The two primers MF (5´-CGAGTCCTTATGAGCTTGATTCTT-3´) and MR (5'-GCCTTCCAGAAGTCGTTTGTTTTC-3') were used according to (Mitra *et al*.,1995). Polymerase Chain Reaction (PCR) amplifications were carried out in a 25-µl reaction volume containing 100 ng of template DNA and 2 µl of each 10 µM primer. To reduce the possibility of cross contamination and variation in the amplification reactions, master mixes containing all PCR reagents including the Kapa Taq polymerase enzyme (KAPA Biosystems, Boston, MA, USA) except DNA template were used. The amplification program was performed using the Gene Amp 9700 thermocycler (Applied Biosystems, Warrington, UK). The amplification protocol was an initial denaturation step for 2 min at 94°C, followed by 35 cycles of 94°C denaturation step for 30s, 58°C annealing step for 30s and 72°C extension step for 30s min. The final step was an extension step at 72°C for 5 min. Electrophoresis of the PCR products was done using 1% agarose gel and bands were detected by UV lamp after syber safe staining using gel documentation system (Amersham Biosciences, Uppsala, Sweden).

DNA Sequencing and Sequence Analysis

PCR products of prolactin was cleaned and sequenced at the Advanced Genetic Technologies Center (http:// www.uky.edu/ Centers/AGTC/). The DNA sequences were edited and aligned using BioEdit software (Hall *et al*., 1999); (Hall http:// www.mbio.ncsu.edu/Bioedit/ bioedit.html). TheBioEdit software was also used to detect SNPs and indel mutations. The BLAST algorithm was used to search the NCBI GenBank database for homologous sequences (http:// www.ncbi.nlm.nih.gov/).

RESULTS AND DISCUSSION

The amplification of the PRL exon 4 generated a PCR product of 156 bp in length from 41 Najdi and 36 Naeimi sheep breeds. To confirm if there are genuine single nucleotide length polymorphisms between the exogenous and indigenous sheep breeds, a genetic map was developed based on different PRL sequences of retrieved from literature or GenBank database e.g. KC764410.1, EU256165.1,EU256166.1, EU256167.1, EU256168.1 ,BC148124.1, JF826522.1, AY339391.1 and JF894307.1and BioEdit software was used to align these sequences. The aligned DNA sequences of PRL region showed no SNPs in the

Table 1. Allele frequencies of the 3 SNPs of PRL region detected in Saudi sheep

Breed/Allele	C05A		G109C		G127T	
	C	A	G	C	G	T
Najdi	0.98	0.02	0.98	0.02	0.98	0.02
Naeimi	1.00	00	1.00	0.00	1	0.00

exon 4 region in Naeimi sheep breed . However, three SNPs (C05A, G109C and G127T) were detected in the exon 4 region. All the detected mutations were only recorded in Najdi sheep. The frequencies of the A , C and T alleles in positions 05, 109 and 127, respectively, were represented only in Najdi sheep breed (Table 1) with frequency of 0.025% for them. When the three SNPs were used

Table 2. Genotypes of the 3 SNPs of PRL region detected in Saudi sheep

Breed	C05A Genotypes			G109C Genotypes			G127T Genotypes		
	CC	CA	AA	GG	GC	CC	GG	GT	TT
Najdi	40	01	00	40	01	00	40	01	00
Naeimi	36	00	00	36	00	00	36	00	00
Total	76	01	00	36	01	00	76	01	00

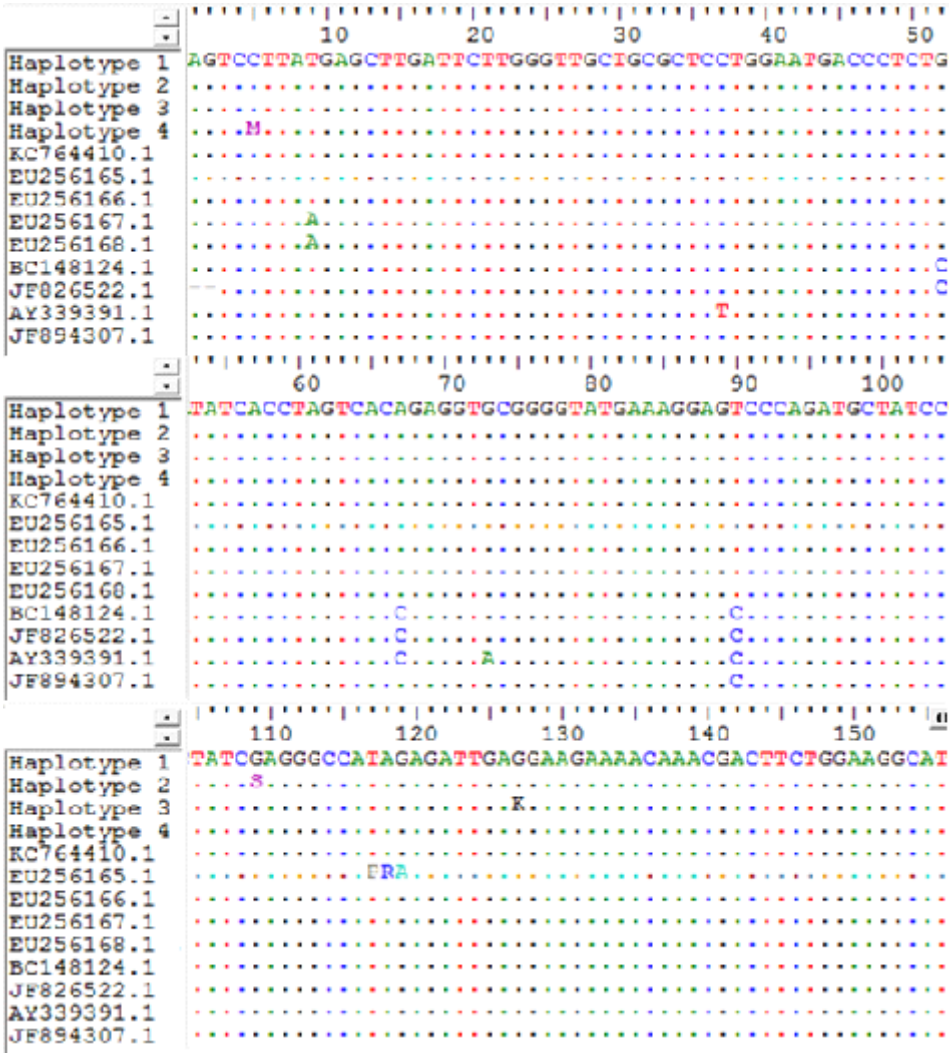

Fig. 1. Alignment of PRL sequences generated from sheep of this study (haplotype1, 2, 3 and 4) and other animals (retrieved from GenBank database). Identical sequences is represented by dots and polymorphism represented by the corresponding one-letter symbol of nucleotides.

to construct different haplotypes, four haplotypes were recovered, H1 (CGG), H2 (CGT), H3 (AGG) and H4 (CCG). The H1 (CGG) was the most common haplotype and found in Najdi and Naeimi individuals with a frequencies of 0.927% and 1.00% for Najdi and Naeimi sheep breeds, respectively. The haplotypes H2, H3 and H4 were unique for Najdi sheep with frequency of 0.025% for each. The genotypes of the three SNPs detected in PRL region in Saudi sheep breeds are shown in (Table 2).

Based on H1, H2, H3 and H4 haplotypes, four different genotypes, one homozygous (genotype 1) and three heterozygous (genotype 2 genotype 3 and genotype 4) were observed in the present study. Genotype 1 was the most frequent genotype and represented by 74 animals, including 38 Najdi and 36 Naeimi followed by genotype 2, genotype 3 and genotype 4 were represented by 1 Najdi sheep for each one of them. The *PRL* gene of exon 4 sequences in Saudi sheep searched against other PRL sequences deposited in NCBI-GenBank database (Figure 1).

The representative *PRL* gene of exon 4 from sheep that deposited in GenBank under accession no.(KC764410.1 and EU256166.1) was identical to the most frequent haplotype 1 recovered from the indigenous Najdi and Naeimi sheep breeds. In the present study the SNPs (C, G and G) at the positions 05,109 and 127, respectively, have been also found in Capra hircus under accession no. (EU256165.1, EU256166.1, EU256167.1, EU256168.1) ,BosTarus under accession no.(BC148124.1, JF826522.1 and AY339391.1) and Bubalus bubalis under accession no.(JF894307.1).we have searched the GenBank database and the results revealed that no polymorphisms have been found for the ovine *PRL* gene. However, only one report in Chinese Hu sheep had detected a single nucleotide polymorphism at exon 4 of *PRL* gene. Mahmoud *et al*.2014 reported that no single nucleotide polymorphism (SNP) detected in the 156-bp PCR amplicon of *PRL*genein Herri sheep.

Only a few studies have been conducted to investigate the association between prolactin gene polymorphism and milk traits in sheep (Ramos *et al*., 2009; Staiger *et al*., 2010). Staiger *et al*.2010 stated that PRL genotype had a significant effect on milk yield. Ewes carrying one allele produced 110.6 g more milk per day than ewes with the A alleles. The increase in production associated with the A allele (110 g of milk/d) has economic relevance because it represents about 7% of the average test-day milk in the study population. Several DNA polymorphisms have been found within the bovine *PRL* gene. Lewin *et al*. 1992 found a silent A→G mutation in codon 103 of exon 4, resulting in a polymorphic RsaI restriction site. They also showed that this PRL-RsaI locus affected milk production traits, including milk yield, milk fat yield and milk protein yield. A subsequent study reported that Holstein-Friesian cows with the GG genotype at PRL-RsaI had a significantly higher milk yield and produced milk with a higher fat percentage than those with the AA genotype (Chung *et al*., 1996). Nucleotide sequence polymorphism was identified within exon 4 of the bovine prolactin gene (Brym*et al*. 2005). Mehmannavaz *et al*. 2009 found the effects of prolactin SNP on genetic trends and the difference between genetic trends produced by two the alleles were not significant for all studied traits in Iranian Holstein bulls. Dybus *et al*. 2005 analyzed the associations between polymorphism localized in the third exon of the prolactin gene (PRL-Rsa1) and milk production traits of Black-and-White and Jersey cattle. They found no associations between *PRL* gene and milk production traits for Black-and White cattle. traits in Iranian Holstein bulls. Kaplan and Boztepe. 2010 determined the prolactin gene (PRL-Rsal) polymorphism within Indigenous Anatolian Water Buffalo breed and Brown Swiss cattle, the results showed monomorphic at exon 3 (PRL-Rsal) loci in Indigenous Anatolian Water Buffalo, prolactin gene mutation in exon 3 Rsal digestion site is not observed in 45 Indigenous Anatolian Water Buffalo breed. This result is identical to the results have been reported by (Mitra *et al*., 1995) These researchers who carried out the study about exon3 PRL-Rsal loci in Murrah, Nili Ravi and Egypt buffalo breed stated that they have observed mutations in Murrah and Nili Ravi buffalo breed, whereas they have not observed any mutation in Egypt buffalo breed.

CONCLUSION

The present study provided basic information to understand the genetic diversity of

local sheep breeds in Saudi Arabia. Sequence analysis revealed three SNPs in exon 4 of the *PRL* gene. Further work is needed to clone the PRL PCR amplicon of the expected heterozygotes to determine the two alleles of this gene. Also, larger samples from each sheep breed are needed to be examined in order to investigate the impact of DNA polymorphisms on prolactin activity and function.

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