The Polymorphism of Pituitary Factor 1 (POU1F1) in Cattle

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Abstract

The development and function of mammary gland is mainly controlled by growth hormone and prolactin, two protein hormones secreted by the anterior pituitary gland. Their synthesis is under regulatory influence of pituitary factor 1 (PIT1 or POU1F1), a protein factor produced in hypothalamic nuclei. In cattle, it was shown that a *Hinf*I polymorphism located in exon 6 of PIT1 gene may have significant influence on milk quantity. In particular A allele was associated with a higher milk yield and could be a valuable genetic marker for improving milk quantity in cattle. In an effort to better understand the possible influence of this polymorphism on mammary gland development and function in cattle, we have studied the frequency this polymorphism in Romanian Black and White breed, a high milk production cattle breed versus Romanian Grey Steppe breed, a primitive breed with very low milk production. In both breeds the frequency of B allele is much higher as compared with the frequency of A allele. The study of PIT1 polymorphism in Romanian cattle breeds is a part of a more complex study targeting several key genes involved in mammary gland function

Keywords: cattle, milk, polymorphism, PIT1, Romanian Grey Steppe, Romanian Black and White

1. Introduction

In the last years the understanding of the genetic basis of mammary gland development and function received an increased attention, because the improvement of milk production should not compromise animal's health [1]. The variations in milk production cannot be attributed just to one gene because the secretory activity of mammary gland is controlled by a cascade of hormones, transcription factors, enzymes, affected by mutations over the years, which are probably the cause of these variations.

The development and function of mammary gland is mainly controlled by growth hormone and prolactin, two protein hormones secreted in the anterior pituitary gland. Their synthesis is under regulatory influence of pituitary factor 1 (PIT1 or POU1F1) [2], a key transcription factor produced in hypothalamic nuclei, which is essential for pituitary gland development and function.

The PIT1 gene is located on cattle chromosome pair 1 [3], coding for a protein composed of 291 amino-acids, containing a DNA binding POU domain [4]. PIT1 is also involved expression of gene coding for thyrotropin releasing hormone (TSH) [5], a key hormone involved in thyroid gland activity.

The inhibition of PIT1 synthesis leads to a marked decrease of growth hormone, prolactin and thyrotropin releasing hormone synthesis [6] and therefore is considered a highly valuable genetic marker for improving milk production [1, 7].

In cattle PIT1 locus several polymorphisms were identified. The first polymorphism, identified in exon 6, is characterised by a substitution of an adenine with a guanine (A207G) located in *Hinf* I restriction site, characterizing the A and B alleles respectively [8].

In Holstein breed it was shown that A allele, characterized by the lack of *Hinf*I restriction site from exon 6 of PIT1 gene, has significant positive

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influence on milk quantity [7, 9], protein yield, body depth, angularity, and rear leg set and could be a valuable genetic marker for improving milk quantity in cattle [7]. In Polish Black and White breed was shown that A allele has a positive influence on some milk production traits [10]. The positive influence of A allele on milk yield was also observed in Romanian Simmental and Maramures Brown cattle breeds [11].

Other studies reported no associations between PIT1-*Hinf*I polymorphism and milk production traits [12, 13].

In American Holstein a cytosine/adenine (C577A) polymorphism located exon 3, leading to a Pro76His substitution in mature protein, was associated with a significant influence on milk yield and productive life [14].

Several other polymorphisms were identified in PIT1 locus: one located in exon 2 [16], two located in intron 3, one in intron 4 and one in intron 5 [17].

Generally the quantity of lactogenic tissue is directly correlated with milk production and therefore the size of mammary gland can offer important clues about its lactating capacity.

Several „actors'' contribute to the lactogenic capacity of mammary gland, PIT1 being on the top of the pyramid through its key role in regulation of expression activity of growth hormone and prolactin genes.

Primitive cattle breeds are much less productive in terms of milk yield than specialized breeds. Therefore they can be used as an excellent model to study the genetic basis of the reduced lactating capacity of mammary gland, compared with highly productive breeds.

In an effort to better understand the possible influence of several key genes polymorphism on mammary gland development and function in cattle [1], the aim of this paper was to comparatively study the frequency PIT1-*Hinf*I polymorphism located in exon 6, in a high milk production cattle breed, as is Romanian Black and White (RBW), Holstein type, and an ancestral breed with very low milk production, as is Romanian Grey Steppe (RGS), which seems to be the closest relative to extinct auroch [15].

2. Materials and methods

Experimental design and blood samples collection.

Initially 24 cattle individuals were chosen for amplification and sequencing of PIT1 exon 6: 16 belonging to the Romanian Black and White breed (RBW), Holstein type, 8 being top milk production individuals (over 12000/lactation) and 8 low milk production individuals. As low milk production breed (about 1200l/lactation), 8 individuals belonging to Romanian Grey Steppe breed (RGS) were chosen for this study.

In order to determine the frequency of *Hinf*I polymorphism from exon 6 on larger populations, an additional number of individuals, 52 cattle from each group (156 in total) were sampled. The blood samples were collected from the jugular vein on K-EDTA anticoagulant. The samples were transported and stored at 4°C.

DNA extraction.

The DNA extraction was performed using Fast to tissue PCR kit (Fermentas, Vilnius, Lithuania) according to manufacturer's instructions, with some modifications as follows: 200 µl of whole blood were washed with PBS solution. The mix was then centrifuged at 14,000g for 30s. The supernatant was removed, the leukocytes pellet was saved and the washing procedure was repeated three times. A master mix of lysis solution was prepared containing 100 µl of lysis buffer and 10 µl Proteinase K (20mg/ml). On hundred and ten microliters of this mix was added to each tube containing the leukocyte pellet. After a short vortexing and centrifugation the samples were incubated at 37^0C for 10 minutes and then for 20 minutes at 55^0C. Inactivation of Proteinase K was done by heating the samples at 95^0C for 3 minutes and by adding 100 µl of neutralisation solution. After a short homogenization and centrifugation, DNA quantity, quality and purity of each sample was assessed by gel migration and by Nanodrop ND 1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

DNA amplification and sequencing.

A 451 base pairs (bp) product, containing a part of PIT1 exon 5 and the entire coding region of exon 6 was amplified from the 180 samples, including the A207G substitution characterizing the A and B

allele. This region was amplified using a set of two primers a forward primer located on intron 5: 5'- AAACCATCATCTCCC TTCTT- 3' and a reverse primer located on exon 6 in 3' untranslated region: 5'- AATGTACAAT GTGCCTTCTGAG-3' [8].
The amplification was performed using direct tissue master mix (Fermentas, Vilnius, Lithuania), 100 µg of genomic DNA, 10 pmol of each primer and the following amplification conditions: 94°C/3 min, followed by 35 cycles of 94°C/60s, 59°C/60s, 72°C/60s and a final extension step at 72°C/7 min.
The amplification products were analyzed in 1,5 % agarose gel containing 1X Sybr Safe (Invitrogen, Eugene, OR, USA). Electrophoresis was performed in TBE buffer (pH= 8.5) at 65 V constant current for 2 hours. The gel was then analyzed with a Molecular Imager Gel Doc XR System (BioRad Laboratories, Hercules, CA, USA).
A number of twenty four amplification products (eight of each cattle group), exhibiting a specific 451 bp product form PIT1 gene, were further submitted to sequencing. The fragments were purified from each reaction using ZR-96 DNA Clean & Concentrator™-5 kit (Zymo Research Corporation, Orange, CA, USA). The sequencing reaction was performed according to the didesoxynucleotide sequencing chain termination method, using BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Both strands were sequenced with the same forward and reverse primers used for PCR amplification. Sequencing products were analyzed by Capillary Electrophoresis on an Applied Biosystem 3730 (Applied Biosystems, Foster City, CA, USA).

PCR-RFLP genotyping of A207G polymorphism from PIT1 exon 6.
The restriction of the remaining samples was performed on 10 µl PCR product with 20,2 *Hinf*I Fast digest mix (Fermentas, Vilnius, Lithuania) containing 1,2µl *Hinf*I, 2µl fast digest mix and 17µl miliQ water. The samples were incubated at 37°C for 30 min and then were analyzed in 3.5% agarose gel as described above.

3. Results and discussion

The sequencing of the 451 bp PCR products, amplified from the PIT1 gene, revealed the A207G substitution from exon 6 characterizing the A and B alleles respectively (Figure 1).

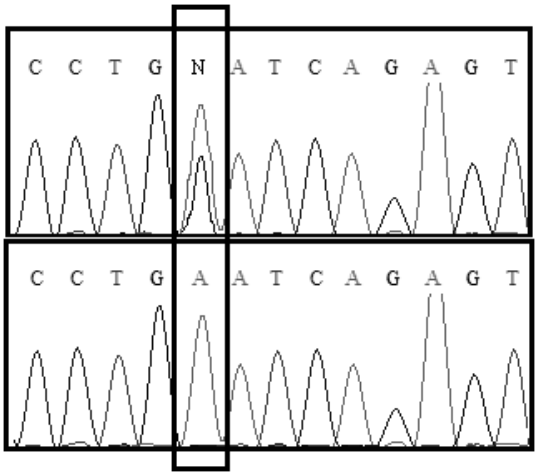

Figure 1. Part of sequencing chromatograms evidencing the A207G (marked by N in upper chromatogram), characterizing A and B allele respectively in cattle
Upper chromatogram: AB genotype
Lower chromatogram: BB genotype

Sequencing revealed the presence of two genotypes AB and BB issued by A207G substitution. The 451 bp fragment amplified from B allele has one restriction site for *Hinf*I enzyme, two fragments being issued following digestion: 244bp and 207bp respectively. The A207G substitution abolishes the *Hinf*I restriction site and as a consequence an additional 451 bp uncut fragment belonging to A allele was observed in heterozygous AB individuals (Figure 2).

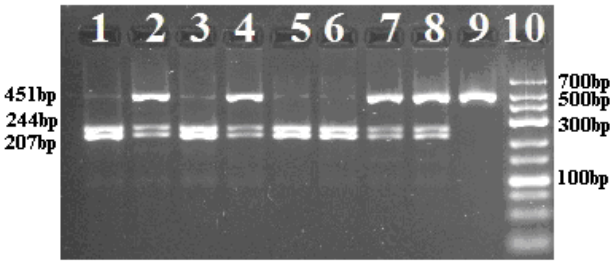

Figure 2. Electrophoresis profile evidencing a *Hinf*I polymorphism located in exon 6 of PIT1 gene. Lanes 1, 3, 5 and 6 - BB genotypes; Lanes 2, 4, 7, 8 - AB genotypes; Lane 9 uncut PCR product; Lane 10: low range DNA ladder (Fermentas, Vilnius, Lithuania)

PCR-RFLP genotyping of the additional samples was in accordance with sequencing data and

revealed just two genotypes BB and AB in the three cattle groups. No AA genotypes were indentified.

The alleles and genotypes frequencies were calculated in the three cattle groups. The frequency of BB genotype was higher in RBW-high milk production individuals and RBW-low milk production individuals, as compared with AB genotypes; in RGS the AB and BB genotypes had a similar frequency (Table 1).

Table 1. Genetic structure in PIT1 locus in analysed populations belonging to RBW and RGS cattle breeds

Breed/group	Number of individuals	Genotype frequency			Allele frequency	
		AA	AB	BB	A	B
RBW[1]-high milk production individuals	60	0	0.182	0.818	0.091	0.909
RBW-low milk production individuals	60	0	0.200	0.800	0.100	0.900
RGS[2]	60	0	0.500	0.500	0.250	0.750

[1]Romanian Black and White
[2]Romanian Grey Steppe

The B allele has a very high frequency as compared with A allele in all three cattle groups. Surprisingly the highest frequency of A allele was observed in RGS breeds (0.250) and the lowest in RBW-high milk production individuals (0.091). This is very intriguing since A allele, associated in Holstein breed with high milk production, is found in RBW with a very low frequency. The frequency of A allele observed in RBW (Table 1) is lower than that observed in other Holstein type populations as Italian Holstein: A=0.19, B=0.81 [7], American Holstein: A=0.15, B=0.85 [9], Iranian Holstein: A=0.21, B=0.85 [13], Polish Black and White A=0.24, B=0.76 [12].

In RGS, an ancestral breed with very low milk production, the higher frequency of A allele (0.250) as compared with RBW (0.091-0.100) is even more intriguing. This suggests that even if PIT1 A allele was associated with a higher milk production, a single mutation in one gene cannot explain the variability of milk production, which is a polygenic trait. In Podolica breed from southern Italy, which belong to the same Podolic cattle group as RGS, the frequency of A and B alleles were pretty close with those observed in RGS: A=0.30, B=0.70 [18].

The higher frequency of A allele suggests that A207G substitution occurred in primitive cattle breeds and was spread in modern cattle subsequently.

4. Conclusions

The present work had as a goal to study a polymorphism of PIT1 gene located in exon 6, associated in several studies with some milk production parameters.

This polymorphism was studied in a high milk production cattle breed, as is RBW (Holstein type) versus a primitive breed with very low milk production, as is RGS. The frequencies A and B alleles and corresponding genotypes were calculated.

The polymorphism of GH in the two Romanian cattle breeds is a part of a more complex study, which is trying to better explain the genetic basis of milk production variability in cattle and goats, by studying the polymorphism of several key genes involved in mammary gland development and its lactation capacity.

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