

Full Length Research Paper

Associations between prolactin gene polymorphism and milk production in montebeliard cows

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Prolactin (PRL) is one of the multi-purpose hormones of the pituitary gland in terms of biological actions especially milk production. In this study associations were analyzed between polymorphisms of the prolactin gene (*PRL-RsaI*) and milk production traits of Montebeliard cows. PCR-RFLP method was used for identification of genotypes. Frequencies of genotypes were 0.81, 0.15 and 0.04 for A/A, A/B and B/B, respectively. The frequency of PRL A allele is 0.89. The results show AA cows yielded most milk in compared of other groups.

Key words: Prolactin gene, montebeliard cows, PCR-RFLP.

INTRODUCTION

Genes influencing polygenic traits characterizing milk or meat production are difficult to identify. However, a number of potential candidate genes have been recognized. They may be selected on the basis of a known relationship between physiological or biochemical processes and production traits and can be tested as quantitative trait loci (QTLs) (Oprzadek et al., 2003).

Prolactin (PRL) is one of the multi-purpose hormones of the pituitary gland in terms of biological actions. More than 100 different and distinct effects of the hormone have been documented. This hormone consists of 197-199 amino acids in most mammalian species (Sinha, 1995). Bovine PRL consists of 199 amino acids (Wallis, 1974). Prolactin is necessary for the initiation and maintenance of lactation. It acts on mammary alveoli to promote the synthesis and secretion of milk protein. This hormone is, primarily responsible for the synthesis of milk proteins, lactose and lipids, all major components of milk (Leprovost et al., 1994). PRL secretion is maintained during lactation by suckling, the most powerful natural stimulus for PRL release (Murai and Ben-jonathan, 1987) PRL regulating reproductive and immunological functions fluid

balance, cellular growth and differentiation (Nicoll, 1982; Loretz and Bern, 1982; Russell, 1989; Kelly et al., 1991). Prolactin (PRL) gene is expressed in the pituitary gland, and at several other sites including the central nervous system, the immune system and the mammary gland (Sinha, 1995, Ben-Jonathan et al., 1996; Leprovost et al., 1994).

Bovine prolactin (PRL) gene is localized in chromosome 23 (Barendse et al., 1997) and consists of five exons separated by interval introns (Camper et al., 1984). Cowan et al. (1990) detected a polymorphic site for *Avall* restriction endonuclease. Digestion of genomic DNA with the restriction endonuclease *Avall* disclosed a probable insertion/deletion of approximately 200 base pairs near the *PRL* gene (Cowan et al., 1990). In the studies carried out by Hart et al. (1993) showed a four-allele SSCP in the 5'- flanking region of the *PRL* gene (Hart et al., 1993). Klauzinska et al. (2001) reported the single nucleotide substitutions in the 5' region of the *PRL* gene. One of these variable sites are also potential binding site for C/EBP transcription factor.

A silent A-G transition mutation at the codon for amino acid 103 in exon 3 of bovine *PRL* gene gives rise to a polymorphic *RsaI* site (Lewin et al., 1992). Chung et al. (1996) showed that *PRL-RsaI* locus had a significant effect on milk yield and fat percentage in dairy cattle (Chung et al., 1996). In the study carried out by Dybus (2002) showed

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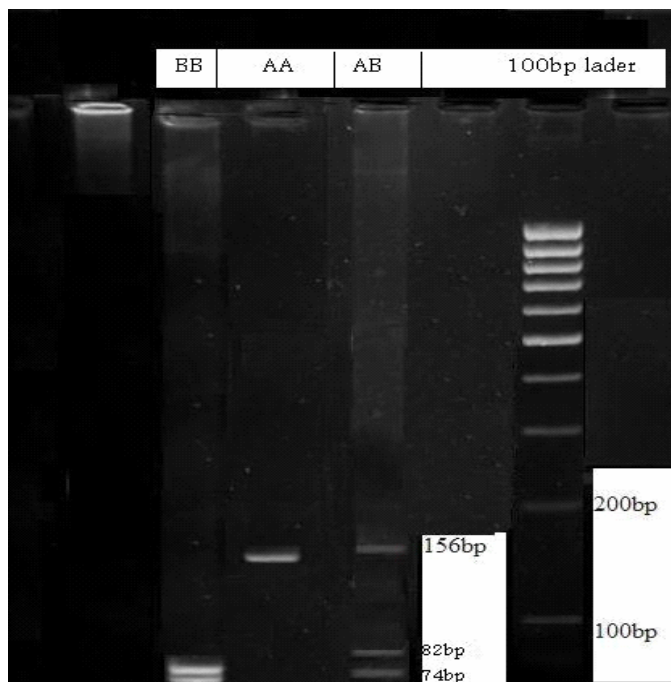


Figure 1. Restriction analysis of *PRL* 156-bp PCR products digested with *RsaI* on 2% agarose gel electrophoresis stained with ethidium bromide. A = undigested 156-bp PCR product; AA genotype; restriction fragments of 82 and 74 bp; BB genotype; restriction fragments of 156, 82 and 74 bp; AB genotype.

that cows with the AA genotypes of the *PRL* gene had higher milk protein content than AB individuals (Dybus, 2002). The aim of this study was to determine the allelic frequencies at the *PRL-RsaI* locus and to investigate the relationship of this polymorphism and milk production traits of Montebeliard cows.

MATERIAL AND METHODS

One-hundred-and-twenty Montebeliard cows were studied. The cows were kept in the Behkede-e-Razavi located at Northern Khorasan in Iran. Blood samples for DNA extraction were collected from the tail vein by venject tube containing EDTA and stored at 20°C. The extraction of DNA from whole blood was performed by phenol-chloroform method.

The 10 months milk production of the cows after first delivery reports by measuring daily milk production of them. The *PRL-RsaI* genotypes were analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A 272 bp fragment of intron 3 of *PRL* gene was amplified by PCR using forward (5'-CGAGTCCTTATGAGCTTGATTCTT-3') and reverse (5'-GCCTTCCAGAAGTCGTTTGTTC-3') primers (Dybus et al., 1995).

The following cycles were applied: denaturation – 94°C/5 min, followed by 30 cycles: denaturation – 94°C for 30 s, primer annealing – 55°C for 1 min, PCR products synthesis – 72°C for 1 min, and final synthesis – 72°C/5 min. Amplified DNA was digested by *RsaI* enzyme at 37°C for 2 h with the following reaction mixture: PCR product 15 µl, buffer 2.5 µl, *RsaI* 0×5 µl, and dH₂O 2 µl. The digestion products were separated by electrophoresis in 2% agarose gels in 1 × TBE and 1×0 µM ethidium bromide. Moreover,

the mean of total milk secretion during 10 months after first pregnancy were evaluated, which was compared with their genotype.

The test for Hardy-Weinberg equilibrium was performed with chi-square. The effect of *PRL* genotypes on the milk production traits of cows were analyzed using SAS and MATLAB softwares.

RESULTS

The following DNA restriction fragments were obtained for the *PRL-RsaI* polymorphism: 82 and 74 bp for the BB genotype, 156, 82 and 74 bp for the AB and 156 bp (no digestion) for the AA. Frequency of allele A and B was 0.89 and 0.11 respectively (Figure 1).

The AA genotype was found the most frequent in the studied herds (0.81), followed by the heterozygotic AB (0.15), whereas the BB was the least frequent (0.04). The population was in equilibrium at these restriction sites using Hardy-Weinberg equilibrium.

The results showed that the AA genotype group yielded most milk in compared of other groups. The mean of total milk production after first delivery in cows with AA genotype was 5805 litter, and it was 4800 litter in AB genotype and 4835 in BB genotype. The difference between these three genotype according to the mean of their total milk production was significant. The mean of total milk production of AA, AB and BB genotype was reported in Table 1.

Table 1. Determination of the relation between PRL genotyping and milk production in Montebeliard cows.

Milk production genotype	Frequency of genotype	Mean of milk production (lit)
AA	0.81	5805
AB	0.15	4800
BB	0.04	4835

DISCUSSION

In dairy cattle, the primary goal of the selection is the improvement of yield and composition of milk. With the advances in molecular biology, the identification of the genes underlying livestock production traits – called quantitative trait loci (QTL) – is now possible and is likely to lead to more efficient breeding programs (Parmentier et al., 1999). Although a number of strategies can be envisaged to identify candidate genes markers, essentially two approaches are presently developed to estimate the favourable QTL implicated in a specific production trait.

Frequencies of *PRL-RsaI* alleles obtained in this study were 0.89 and 0.11. Higher frequency of the *PRL* (0.95) in Holstein breed was observed by others (Chrenek et al., 1999). Somewhat smaller frequency of the *PRL* (0.80) was observed in other studies (Mitra et al., 1995). Considerably smaller frequencies of *PRL* (0.73) were observed by others (Chung et al., 1996).

Influence of the *PRL-RsaI* polymorphism on milk production traits was seen in the analyzed Jersey cows. The statistically significant differences between individuals of different *PRL* genotypes were found in milk fat yield, milk fat content and sum of fat and protein content. In the first 305-day lactation, the cows of the *AA* genotype produced less milk fat (-15.9 and -14.6 kg) than the *AB* and *BB* individuals (Brym et al., 204). Different results were obtained by Chung et al. (1996) who reported that Holstein-Friesian cows with *AA* genotype produced milk with higher fat content than *BB* individuals. Chrenek et al. (1999) examined an influence of *PRL-RsaI* polymorphism on the milk production traits of Brown Swiss cows, and there were no statistically significant differences between the cows with different *PRL* genotype. Dybus et al. (2005) reported that no associations between RFLP in *PRL* gene and milk production traits but the result of this study was the *AA* genotype group yielded most milk in compared of other groups.

In conclusion, the Montebeliard cows in the Behkedeh-e-Razavi located at Northern Khorasan in Iran are the only group of these type in Iran and by the high milk production recorded of *AA* genotype, mating of these cattle should be consider for other low milk produced cows.

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REFERENCES

Barendse W, Vaiman D, Kemp SJ, Sugimoto Y, Armitage SM, Williams JL, Sun et al. A (1997) Medium-Density Genetic Linkage Map Of The Bovine Genome. *Mamm. Genome* 8:21-28.

Ben-Jonathan N, Mershon JL, Allen DI, Steinmetz RW (1996) Extrapituitary Prolactin: Distribution, Regulation, Functions, And Clinical Aspects. *Endocr. Rev.*, 17: 639-669.

Camper SA, Luck DN, Yao Y, Woychik RP, Goodwin RG, Lyons RH, Rottman FM (1984) Characterization of The Bovine Prolactin Gene. *Dna* 3:237-249.

Chrenek P, Huba J, Oravcova M, Hetenyi L, Peskovieova D, Bulla J (1999) Genotypes Of Bgh And Bprl Genes In Relationships To Milk Production. *Eaap – 50th Annual Meeting, Zurich, Book of Abstracts*, 40.

Chung ER, Rhim TJ, Han SK (1996) Associations Between Pcr-Rflp Markers Of Growth Hormone And Prolactin Genes And Production Traits In Dairy Cattle. *Korean J. Anim. Sci.* 38: 321-336.

Cowan CM, Dentine MR, Ax RL, Schuler LA (1990) Structural Variation Around Prolactin Gene Linked To Quantitative Traits In An Elite Holstein Sire Family. *Theor. Appl. Gene.* 79: 577-582.

Dybus A (2002). Associations Of Growth Hormone (Gh) And Prolactin (Prl) Genes Polymorphisms With Milk Production Traits In Polish Black-And-White Cattle. *Anim. Sci. Papers Reports.* 20:203-212.

Hart GL, Bastiaansen J, Dentine MR, Kirkpatrick BW (1993). Detection Of A Four-Allele Single Strand Conformation Polymorphism (Sscp) In The Bovine Prolactin Gene 5' Flank. *Anim. Gene.* 24:149.

Kelly PA, Djiane J, Postel-Vinay MC, Ederly M (1991). The Prolactin/Growth Hormone Receptor Family. *Endocr. Rev.* 12:235-251.

Leprovost F, Leroux C, Martin P, Gaye P, Djiane J (1994) Prolactin Gene Expression In Ovine And Caprine Mammary Gland. *Neuroendocrinology* 60:305-313.

Lewin HA, Schmitt K, Hubert R, Van Eijk MJ, Arnheim N (1992). Close Linkage Between Bovine Prolactin And Bola-Drb3 Genes: Genetic Mapping In Cattle By Single Sperm Typing. *Genomics* 13: 44-48.

Loretz CA, Bern HA(1982). Prolactin And Osmoregulation In Vertebrates. *Neuroendocrinology* 35: 292-304.

Mitra A, Schlee P, Balakrishnan CR, Pirchner F (1995). Polymorphisms At Growth-Hormone And Prolactin Loci In Indian Cattle And Buffalo. *J. Anim. Breed. Gene.* 112:71-74.

Murai I, Ben-Jonathan N (1987). Posterior Pituitary Lobectomy Abolishes The Suckling-Induced Rise In Prolactin (PRL): Evidence For A PRL Releasing Factor In The Posterior Pituitary. *Endocrinol* 121: 205-211.

Nicoll CS (1980). Ontogeny and evolution of prolactin's functions. *FASEB J.* 39:2563-2566.

Oprzadek J, Flisikowski K, Zwierzchowski L, Dymnicki Z (2003). Polymorphism At Loci Of Leptin, Pit1 And STAT5A And Their Association With Growth, Feed Conversion And Carcass Quality In Black-And-Whith Bulls. *Anim. Sci Paper Reports*, 21:135-145.

Parmentier I, Portetelle D, Gengler N, Prandi A, Bertozzi C, Vleurick L, Gilson R, Renaville R (1999). Candidate Gene Markers Associated With Somatotropic Axis And Milk Selection. *Domest. Anim. Endocr.* 17:139-148.

Russell DH (1989). New Aspects Of Prolactin And Immunity: A Lymphocyte-Derived Prolactin Likes Product And Nuclear Protein Ki-

nase C Activation. Trends iFarmacol. Sci. 10:40-44.
Sinha YN (1995) Structural Variants Of Prolactin: Occurrence And
Physiological Significance. Endocrine Rev, 16: 354-369

Wallis M (1974). The Primary Structure of Bovine Prolactin. Fed. Euro.
Biochem. Soc. Lett. 44: 205-208