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SHORT REPORT

Polymorphism within the bovine prolactin receptor gene (*PRLR*)*

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In marker-assisted selection (MAS) of dairy cattle certain genes are proposed as potential candidates associated with dairy performance traits. Among different candidates, prolactin receptor gene (*PRLR*) seems to be promising because of its crucial role in transmitting signal from lactogenic hormones to milk protein gene promoters. In this study nine PCR fragments representing most important functional domains of *PRLR* were screened for polymorphism. Using SSCP method one SNP (A→C) was found in intron 9. The SNP was deposited in GenBank AY484400 and AY339393 at position of 205 nt, for Jersey and Polish Black-and-White cattle, respectively. Allele frequency was estimated in 186 Polish Black-and-White (0.981 and 0.019 for *A* and *C*, respectively) and 138 Jersey (0.812 and 0.188 for *A* and *C*, respectively) cows. Preliminary analysis showed no significant associations between *PRLR* genotypes and milk performance traits. However, Jersey cows of *CC* genotype produced more milk with higher protein content than those of *AA* and *AC* genotypes. Because of the low number of cows of *CC* genotype, it is necessary to investigate more numerous population of cattle in which all genotypes will be efficiently represented.

KEY WORDS: cattle / gene polymorphism / prolactin receptor gene / SSCP

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Prolactin receptor (PRLR) is thought to play a central role in signal transmitting from prolactin to milk protein genes. It binds prolactin and contributes to activation of JAK2 kinases and subsequent phosphorylation of STAT5 transcription factors which bind to recognition sequences located in promoters of milk protein genes [Bole-Feysot *et al*. 1998]. Therefore, *PRLR* is suggested as candidate gene associated with milk protein yield and content in dairy cattle. The gene coding for bovine PRLR was mapped on chromosome 20q17 [Hayes *et al*. 1996]. In cattle, there are two distinct prolactin receptor isoforms: long PRLR with 557 amino acids [Scott *et al*. 1992] and short PRLR with 272 amino acids [Schuler *et al*. 1997] produced by alternative splicing mechanism using a single intron with one 5' and two 3' sites [Bignon *et al*. 1997]. The short PRLR is unable to mediate transcriptional activation *via* JAK-STAT pathway. Furthermore, short isoform can inhibit long PRLR activation of JAK2 and transcription *via* formation of heterodimers [Bole-Feysot *et al*. 1998]. So far, no polymorphism was reported for *PRLR* gene in cattle.

In the present study the first nucleotide polymorphism in intron 9 of the bovine *PRLR* gene is described.

Material and methods

Initial screening for polymorphism within bovine *PRLR* gene was performed in individuals of dairy *vs* beef cattle: Polish Black-and-White (n=20), Jersey (n=10), Polish Red (n=6), Simmental (n=3), Limousine (n=3) and Charolaise (n=3). Next, genetic structure of population and possible association(s) of *PRLR* gene variants with milk performance traits were analysed in Polish Black-and-White (n=186) and Jersey (n=138) cattle. Approximately 9 ml blood was withdrawn from each animal. Genomic DNA was isolated from leukocytes by the MasterPure DNA Purification Kit (EPICENTRE).

The following were PCR conditions applied for PRLR 241 bp gene fragment. The PCR was carried out in 25 µl of mix containing: 1.25 µl 20×PCR buffer (EPICENTRE); 1.3 µl dNTP (2 mmol each); 70 pmol of each primer: forward 5' CATGGTGACCTGCATCCTC 3', reverse 5'ACCCTCATGCCTCTCACATC 3'; 1.5 mM $MgCl_2$; 5 µl 10× Enhancer (EPICENTRE); 0,8 U Tfl DNA Polymerase (EPICENTRE); 100-600 ng of genomic DNA and H_20 up to 25 µl. The PCR reaction was carried in EPPENDORF MASTERCYCLER 5330 thermocycler and cycling conditions were as follows: initial denaturation (94°C/3 min), followed by 35 cycles of denaturation (94°C/30 s), annealing (59.4°C/30 s) and extension (72°C/30 s), followed by final synthesis (72°C/5 min).

SSCP analysis was performed according to Kamiński [1998]. PCR products representing different SSCP patterns were sequenced using ABI PRISM™ 377 DNA Sequencer (APPLIED BIOSYSTEMS) and DYEnamic ET Terminator Cycle Sequencing Kit (AMERSHAM BIOSCIENCES). The results were analysed by Staden Package (http://staden.sourceforge.net/).

Frequency of alleles and genotypes and their accordance with Hardy-Weinberg

equilibrium was calculated by POPGENE ver. 1.31 software (http://www.ualberta.ca/~fyeh). Frequency of alleles and genotypes in Jersey and Black-and-White cows were compared by the chi-square test. The effects of *PRLR* genotype on milk production traits of cows were analysed using GLM procedure (STATISTICA 6, StatSoft Inc). The following model was used:

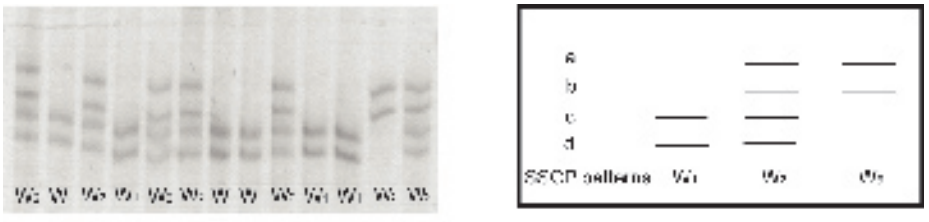
$$Y_{ijk} = \mu + G_i + YS_j + (GYS)_{ij} + e_{ijk}$$

where:
Y_{ijk} – analysed trait of *k*-th cow;
μ – overall mean;
G_i – effect of *i*-th genotype;
YSj – effect of *j*-th year-season of calving;
$(GYS)_{ij}$ – interaction between the main effects of the model;
e_{ijk} – random error.

The following milk performance traits were considered: milk yield (kg), fat yield (kg), fat content (%), protein yield (kg) and protein content (%). Data on milk performance traits for lactation I were retrieved from breeding documentation available from owners of cows.

Results and discussion

Based on the genomic sequences available in GenBank (L02549, AF042780), nine pairs of PCR primers encompassing almost the entire encoding sequences for *PRLR* were designed by PRIMER3 software (www.genome.wi.mit.edu). Nine specific PCR products were obtained (data not shown) and initial screening for polymorphism by SSCP analysis was carried out. SSCP polymorphism was observed only for one PCR product – *PRLR* 241 bp. Three SSCP patterns were observed: W_1, W_2 and W_3 (Fig. 1). Sequencing of these SSCP patterns revealed a single nucleotide substitution $A \rightarrow C$ (Fig. 2) at position of 205 nt, allowing for real genotype designation of *AA, AC* and *CC* for W_1, W_2 and W_3, respectively. The SNP is located in intron 9 (according to human *PRLR* gene sequence AH007727) which is involved in alternative splicing of short



A B

Fig. 1. SSCP patterns *PRLR* 241 bp

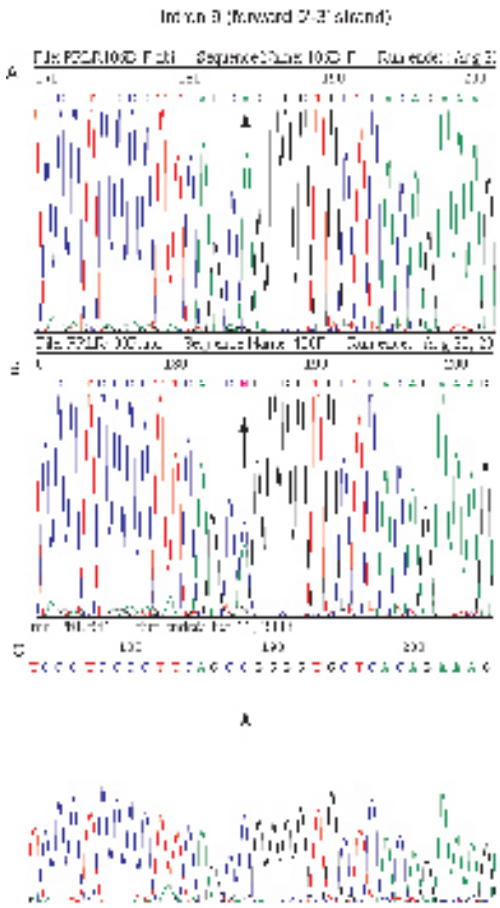


Fig. 2. Direct sequencing of *PRLR* 241 bp PCR products

and long form of prolactin receptor [Bignon *et al*. 1997].

The frequencies of genotypes in Black-and-White and Jersey cows are shown in Table 1. Both populations remained in the Hardy-Weinberg equilibrium. Significant difference (*chi-square* test, P≤0.01) was ascertained between frequency of *AA* and *AC* genotypes between breeds analysed.

Preliminary analysis showed no significant associations between *PRLR* genotypes and milk performance traits, although Jersey *CC* cows yielded more milk with higher protein content (Tab. 2) in the first lactation. In the near future we intend to find more *CC* cows and perform complete statistical analysis.

In MAS of dairy cattle some genes are proposed as potential candidates associated with dairy performance traits. Among different candidates, *PRLR* gene seems to be promising because of its crucial role in transmitting signal from lactogenic hormones to milk protein gene promoters. In the present investigation we assumed that SNPs occurring within *PRLR* gene may affect milk protein biosynthesis and hence chemical composition of milk, or at least be an effective DNA marker of cattle genome region. We screened all exons coding functional domains of PRLR except one encoding transmembrane domain. Using PCR-SSCP method we found only one SNP located in intron 9. The SNP was deposited in GenBank AY484400 and AY339393, for Jersey and Polish Black-and-White cattle, respectively. It is the first polymorphism found and reported in the bovine prolactin gene.

Because the SNP is a silent mutation it cannot be causative mutation affecting any phenotypic variation, and can be suggested as a new marker for a genome region within which it resides. However, it is noteworthy that even silent mutations in introns may turn to be QTLs *loci* as it was proved in pigs [Van Laere *et al*. 2003].

Table 1. Frequency of *PRLR* genotypes and alleles in Black-and-White (*BW*) and Jersey cows

Breed	Genotype number/frequency			Allele frequency	
	AA	*AC*	*CC*	*A*	*C*
BW (n=186)	179/0.962[A]	7/0.038[B]	–	0.981[E]	0.019[F]
Jersey (n=138)	89/0.645[A]	46/0.333[B]	3/0.022	0.812[F]	0.188[F]

[A,B,E,F] Within columns frequencies bearing the same superscript differ significantly at $P \leq 0.01$.

Table 2. Means and their standard deviations (SD) of milk performance traits in Black-and-White (BW) and Jersey cows of different *PRLR* genotypes

Breed	Genotype	Milk (kg)	Fat		Protein	
			(kg)	(%)	(kg)	(%)
BW	*AA* (n=179)	7648,76 (1061,91)	321,63 (54,78)	4,21 (0,55)	242,73 (31,01)	3,18 (0,18)
	AC (n=7)	7366,28 (1132,61)	324, 14 (55,78)	4,41 (0,42)	229,00 (30,11)	3,18 (0,17)
Jersey	*AA* (n=89)	3932,93 (555,39)	221,20 (28,62)	5,66 (0,54)	152,64 (19,78)	3,77 (0,24)
	AC (n=46)	3970,35 (695,49)	217,30 (33,28)	5,51 (0,54)	152,52 (22,77)	3,86 (0,24)
	CC (n=3)	4227,33 (271,32)	240,00 (7,81)	5,70 (0,53)	164,00 (8,88)	3,88 (0,09)

Comparison of allele frequency revealed significant differences between genotype and allele frequencies in both breeds. It may indicate that the SNP is a marker of unknown breed-specific *locus* involved in determining the extreme protein content of milk. However, because of very low number of *CC* cows we could not prove this hypothesis. It is noteworthy that close to prolactin receptor gene the QTL was mapped for protein content of milk [Mosig *et al*. 2001].

It is believed that SNPs located in candidate genes for quantitative traits or close to hypothetical QTL may be very useful in genome scanning of SNPs using high throughput method, *e.g*. microarray genotyping.

REFERENCES

1. BIGNON C., BINART N., ORMANDY C., SCHULER L.A., KELLY P.A., DJIANE J., 1997 – Long and short forms of the ovine prolactin receptor: cDNA cloning and genomic analysis reveal that the two forms arise by different alternative splicing mechanisms in ruminants and in rodents. ***Journal of Molecular Endocrinology*** 19, 109-120.
2. BOLE-FEYSOT CH., GOFFIN V., EDERY M., BINART N., KELLY P.A., 1998 – Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. ***Endocrine Reviews*** 19, 225-268.

3. HAYES H., LE CHALONY C., GOUBIN G., MERCIER D., PAYEN E.J., BIGNON C., KOHNO K., 1996 – Localization of ZNF164, ZNF146, GGTA1, SOX2, PRLR and EEF2 on homeologous cattle, sheep, and goat chromosomes by fluorescent in situ hybridization and comparison with human gene map. ***Cytogenetics and Cell Genetics*** 72, 342-346.
4. KAMIŃSKI S., 1998 – Identification of bovine kappa casein genotypes by the PCR-SSCP method. ***Animal Science Papers and Reports*** 16, 13-17.
5. MOSIG M.O., LIPKIN E., KHUTORESKAYA G., TCHOURZYNA E., SOLLER M., FRIEDMANN A., 2001 – A whole genome scan for Quantitative Trait Loci affecting milk protein percentage in Israeli-Holstein cattle, by means of selective milk DNA pooling in a daughter design, using an adjusted false discovery rate criterion. ***Genetics*** 157, 1683-1698.
6. SCHULER L.A., NAGEL R.J., GAO J., HORSEMAN N.D., KESSLER M.A., 1997 – Prolactin receptor heterogeneity in bovine fetal and maternal tissues. ***Endocrinology*** 138, 3187-3194.
7. SCOTT P., KESSLER M.A., SCHULER L.A., 1992 – Molecular cloning of the bovine prolactin receptor and distribution of prolactin and growth hormone receptor transcripts in fetal and utero-placental tissues. ***Molecular and Cellular Endocrinology*** 89, 47-58.
8. VAN LAERE A.S., NGUYEN M., BRAUNSCHWEIG M., NEZERE C., COLLETTE C., MOREAU L., ARCHIBALD A.L., HALEY CH.S., BUYS N., TALLY M., ANDERSSON G., GEORGES M., ANDERSSON L., 2003 – A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. ***Nature*** 425, 832-836.

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Polimorfizm w genie receptora prolaktyny (*PRLR*) bydła

S t r e s z c z e n i e

Receptor prolaktyny pośredniczy w przekazywaniu sygnału od hormonów laktogennych do promotorów genów białek mleka. Gen receptora prolaktyny (*PRLR*) jest potencjalnym kandydatem na marker cech ilościowych bydła mlecznego. Wykorzystując metodę SSCP analizowano polimorfizm wśród 9 fragmentów PCR, obejmujących prawie całą sekwencję kodującą genu *PRLR*. Zaobserwowano polimorfizm SSCP dla amplikonów *PRLR* 241 pz. Analiza sekwencyjna prób DNA o odmiennych wzorcach SSCP, wykazała istnienie SNP (A→C) w pozycji 205 nt (GenBank AY484400 i AY339393). Wykryta mutacja znajduje się w intronie 9. W analizowanych populacjach bydła rasy cb (n=186) oraz jersey (n=138) zaobserwowano odpowiednio następujące frekwencje alleli: 0,981 i 0,812 dla allelu *A* oraz 0,019 i 0,188 dla allelu *C*. Wstępna analiza nie wykazała istotnych zależności między genotypem *PRLR* a cechami użytkowości mlecznej. Niemniej zauważono, że krowy rasy Jersey o genotypie *CC* charakteryzowały się wyższą wydajnością mleka oraz wyższą zawartością białka w mleku. Obserwacja ta wymaga jednak potwierdzenia na liczniejszej populacji bydła.