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Short Communication

Characterization of PRLR and PPARGC1A genes in buffalo (*Bubalus bubalis*)

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Abstract

More than 40 million households in India depend at least partially on livestock production. Buffaloes are one of the major milk producers in India. The prolactin receptor (PRLR) gene and peroxisome proliferators activated receptor-γ coactivator 1-alpha (PPARGC1A) gene are reportedly associated with milk protein and milk fat yields in *Bos taurus*. In this study, we sequenced the PRLR and PPARGC1A genes in the water buffalo *Bubalus bubalis*. The PRLR and PPARGC1A genes coded for 581 and 819 amino acids, respectively. The *B. bubalis* PRLR gene differed from the corresponding *Bos taurus* at 21 positions and four differences with an additional arginine at position 620 in the PPARGC1A gene were found in the amino acid sequence. All of the changes were confirmed by cDNA sequencing. Twelve buffalo-specific single nucleotide polymorphisms (SNPs) were identified in both genes, with five of them being non-synonymous.

Key words: Bubalus bubalis, buffalo, PRLR gene, PPARGC1A gene.

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The water buffalo (*Bubalus bubalis*) is an important dairy animal in the Indian subcontinent, with 55% of the milk in India being produced by buffaloes. With over 105 million buffaloes (56% of the world's total population), India needs to improve the productivity of buffaloes as this species is rapidly replacing cattle in several milk-producing areas. Many candidate genes have been associated with dairy performance traits. The prolactin receptor (PRLR) gene, which belongs to the hematopoietin receptor superfamily (Kossiakoff *et al.*, 1994), is particularly promising in this regard because of its crucial role in signal transduction from lactogenic hormones to milk protein gene promoters (Hayes *et al.*, 1996). Prolactin (PRL) regulates milk gene expression (Gao *et al.*, 1996) by activating Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5 (STAT5), initially termed mammary gland factor (MGF). The gene coding for bovine PRLR was mapped to chromosome 20 in cattle and chromosome 19 in buffalo (Amaral *et al.*, 2008). This gene has nine exons that code for a polypeptide of 581 amino acids.

The bovine peroxisome proliferators activated receptor-γ coactivator 1-alpha (PPARGC1A) gene is another putative gene associated with a previously described quantitative trait locus (QTL) for milk fat yield on BTA6 (Weikard *et al.*, 2005). The protein encoded by this gene is a transcriptional coactivator that regulates the genes involved in energy metabolism. The bovine PPARGC1A gene is organized into 14 exons consisting of 6261 bp and is expressed at different levels in a large number of tissues.

In this work, we determined the exon sequences of the buffalo PRLR and PPARGC1A genes and confirmed the exon-intron boundaries by cDNA sequencing. We also identified the SNPs in a panel of various buffalo breeds.

The PRLR and PPARGC1A genes were characterized in a panel of 24 animals drawn from six Indian water buffalo breeds (Murrah, Bhadawari, Tarai, Pandharpuri, Marathwada and Mehsana). The Murrah breed is a northern Indian large dairy breed whereas Bhadawari and Tarai are small sized breeds adapted to extensive production systems. The Pandharpuri and Marathwada breeds are medium sized buffaloes from central India and the Mehsana breed is a western Indian dairy breed. DNA was isolated from blood samples and diluted to an optimum concentration. The genomic regions corresponding to the putative buffalo PRLR and PPARGC1A genes were amplified by the polymerase chain reaction (PCR) with 11 primer pairs for PRLR and 17 primer pairs for the PPARGC1A gene designed from the *Bos taurus* GenBank accession nos. AJ966356 and AY321517, respectively, at exon-intron boundaries (Table 1). The amplifications were run in 10 μL reaction mixtures containing 50 ng of genomic DNA, 5 pmol of each primer, 2 mM $MgCl_2$, 0.2 μM of each dNTP and 1 unit of *Taq* DNA polymerase (Sigma) in a thermocycler (icycler Biorad, San Diego, CA, USA). The amplification protocol consisted of 5 min denaturation at 94 °C followed by 35 amplification cycles of 94 °C for 45 s, 55 °C

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Table 1 - Primers used in this work.

Primer name	Forward primer	Reverse primer
PRLR gene		
PRL1	TCTgTTCATggAggCAAATg	AAgCAACAgCAggACAgATg
PRL2	TgTgCCTCACCAgACTTTTg	gggACTgTgATggATTCTCC
PRL3	CCCCATCTACCTgCTTCTgT	AATTAACgCAgggTCAgTgg
PRL4	AgCAAggAAgCTCCATACCA	CggggATCTATCCCTAAgACA
PRL5	AAgCTAgCgAgATCTgCCTCT	TAggAggCACgACTggTTCT
PRL6	gACCTACATACTggCTTCTCTgC	AAAACAATggCAgATTTCAgg
PRL7	ggCAggggACTTATgTTCAA	gAggTgCTTgAATTATCTgTAggTT
PRL8	CATgTAgCAgCCATTTggAA	gCTTAgCCAgACTgCACTgA
PRL9	gCgTTCACTTTgATTgCAgA	TgTTggTCTgTTTggTCCAg
PRL10	CAACATTgCTgACgTgTgTg	CAATTgAACCCATCCTTCCA
PRL11	gTgTCCCgggTgACAgATAg	AAACCATgAAggCCTTTTCC
PPARGC1A gene		
PRGC_1	CATgATgCTCCAAAAgTCCA	CCCCTCACAggAATATTTgC
PRGC_2	TTTTTCTCCCTgCCTCCTg	CAAAgCAAgAACCCATTATgC
PRGC_3	CTCATCTCCCAgTgTCAACTCA	gTAgCCAgAggCAACTCCAA
PRGC_5	AAAgTTTTAgTAgCTTATTCTCATgCT	TCCAgCTgAATTAATACATAgAATCC
PRGC_6	TgAACACTTCATTgAAAATCTCATC	gAAgTTTgggTgTCCTCAgC
PRGC_7	AgTTTTCCTgTTTCCAgTTTCC	ATATACATTTCACATACACACTCATCC
PRGC_8	ggAAAATgTgTCCTggCATT	gCggTCTCTCTCAggTAgCA
PRGC_9	AggAgCTCCATgACTCCAgA	CTTAggCTTTgggTgggTTT
PRGC_10	TCCAggggCTACTCAgTCAT	ggCAgggTTTTggACTACAT
PRGC_11	gCCCCCTCCCTAgTTTATgA	AATCTATgCCCATCACATCC
PRGC_12	TgCCTTCATAAATggTTCTgg	CTgTCAAAgCATTCCCATCC
PRGC_13	gCgAAgTCTCCAAAgTggAA	AgTAATggTTTggCCCAATg
PRGC_14	CggATAAATTgTTTTTgAATCg	TgCAATAgTCTTTTAgggAAggA
PRGC_15	gAAgACACCgCAggTTCTgTA	TTCTCgATTAAgAAAAATTTAgCAg
PRGC_16	TTTgTTACAgCTATgCACTgTAAATg	gCATTTTgTATCAAATTAAAATCACA
PRGC_17	CCATTAAgAAATgTTTTTATTTTCTCT	gAgTgTggAgCCCTggAAT

(variable) for 45 s and 72 °C for 45 s, with a final extension at 72 °C for 10 min. The PCR products were separated on 2% (w/v) agarose (Promega, USA) gels to determine fragment sizes. PCR products were then incubated with exonuclease and calf intestinal phosphatase (1 unit each) at 37 °C for 2 h followed by inactivation at 85 °C for 15 min and subjected to cycle sequencing reactions in a 5 μL reaction volume using the Big-Dye system (Applied Biosystems, USA) according to the manufacturer's instructions. Sequencing was done in a 3100 Avant automated sequencer (Applied Biosystems, USA) using forward and reverse primers.

Total RNA was isolated from buffalo mammary gland tissue using a High pure RNA kit (Roche, Germany). cDNA was synthesized from total RNA using a superscript reverse transcriptase system (RT-PCR) and a first strand cDNA synthesis kit (Invitrogen, New Zealand) according to the manufacturers instructions. Sequencing was done in a 3130xl Genetic Analyser (Applied Biosystems) using forward and reverse primers designed for the coding region.

The sequences obtained for each fragment were aligned with SeqScape software v.2 (Applied Biosystems) to obtain PRLR and PPARGC1A gene consensus sequences using the *B. taurus* sequances as reference. The predicted amino acid sequences were obtained using AnnHyb 4.943 software (Friard, 2010). Single nucleotide polymorphisms (SNPs) were detected in the buffalo PRLR and PPARGC1A genes and confirmed in additional samples.

The PRLR gene sequence of *B. bubalis* consisted of nine exons. Overall, 4338 bp of genomic DNA (GQ339914) and 1239 bp of cDNA (HQ236497) were sequenced. The buffalo PRLR gene encoded 581 amino acids. Comparison with the corresponding *B. taurus* sequence revealed 21 amino acid differences (positions 2, 19, 52, 57, 61, 66, 100, 208, 243, 299, 338, 347, 399, 443, 480, 485, 494, 497, 534, 539 and 548). Six buffalo- specific SNPs were identified in the PRLR exon regions of the *B. bubalis* gene (positions G305T* in exon 2, A1017G in exon 4, and A2690G*, G3009A*, A3221G and A3486G in exon 9). Three SNPs were non-synonymous (*), resulting in amino

Table 2 - Buffalo-specific SNPs identified in the PRLR and PPARGC1A genes.

Gene	Position	NCBI dbSNP accession no.	SNP	Codon change	Region	Amino acid change
PRLR (GQ339914)	305	SS# 410759785	G/T*	GTC/TTC	Exon2	V19F
	1017	SS# 410759786	A/g		Exon4	
	2690	SS# 410759787	A/g*	CAC/CGC		H328R
	3009	SS# 410759788	G/A*	GGC/AGC		G428S
	3221	SS# 410759789	A/g		Exon9	
	3486	SS# 410759790	A/g			
PPARGC1A (GU066311)	718	SS# 410759779	C/T		Intron3	
	1844	SS# 410759780	A/g		Exon6	
	1902	SS# 410759781	C/T		Intron6	
	2382	SS# 410759782	G/T*	GGG/TGG		W346G
	2529	SS# 410759783	C/T*	CTC/TTC	Exon8	F395L
	2657	SS# 410759808	A/g			

*Non synonymous.

acid changes at positions V19F, H328R and G428S (Table 2).

The PPARGC1A gene (GU066311) was sequenced by designing primers at the exon-intron boundaries for most of the exons because the large size of the introns precluded sequencing. However, intron 6 was small enough to be completely sequenced. The amino acid sequence predicted from genomic DNA was confirmed by sequencing the cDNA (HQ236498). The PPARGC1A gene encoded 819 amino acids and comparison with the corresponding *B. taurus* sequence revealed four differences (positions 392, 419, 534 and 774), with the additional amino acid (arginine) at position 620. Six buffalo-specific SNPs were identified in the PPARGC1A gene (positions C718T in intron 3, A1844G in exon 6, C1902T in intron 6, and G2382T*, C2529T* and A2657G in exon 8). Two SNPs were non-synonymous (*), resulting in amino acid changes at positions W346G and F395L (Table 2).

Comparison with the corresponding *B. taurus* genes revealed that in both species the two genes contained an equal number of exons of similar length, except for exon 9 in the PPARGC1A gene, which had three more nucleotides that encoded an additional amino acid (arginine). The sequence conservation and exon-intron splice sites of *B. bubalis* and *B. taurus* were confirmed by sequencing the cDNA of both genes.

In this study, we sequenced and characterized the PRLR and PPARGC1A genes that are putative candidate genes for milk quality and production in buffaloes. We also identified novel buffalo-specific SNPs. Of the 12 SNPs identified, five were non-synonymous, *i.e.*, they can theoretically change protein structure and function. These SNPs may be useful as markers for milk quality and production traits in buffaloes.

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Internet Resources

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