



## Short Communication

# Molecular Effects of Polymorphism in the 3'UTR of Osteopontin Gene in Riverine Buffalo

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## ABSTRACT

Nucleotide sequencing of bovine osteopontin gene showed a novel DNA polymorphism 37421762 (C>A) in the 3'-untranslated region (3'-UTR) of the gene. Association test was performed to analyze its relation with milk composition but it was found non-significant in all analyzed traits. So, it was inferred that it might be due to environmental or epigenetic effect, sampling number or a breed difference. To know the functional consequences of the identified polymorphism, RNA secondary structure analysis was performed and SNP was found changing the termination loop shape in mRNA. However, to increase the selection efficacy of dairy animals, the effects of candidate polymorphism should further be screened in different environments and genetic backgrounds.

## Article Information

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## Authors' Contributions

AN conceived and designed the study. SM, AN, TH did sampling and genome extraction. SM, MI, AW amplified the markers. MJ, MEB, WS, analyzed the data. AN, SM, ASH wrote the article.

## Key words

Polymorphism, 3'UTR, Osteopontin gene, Riverine buffalo.

Osteopontin (SPP1, OPN) is a multifunctional protein involved in a number of biological processes; chemotaxis, bone remodeling, cell adhesion, inhibition of ectopic calcification, fetal growth and development, cancer metastasis and immune modulatory functions (Schack *et al.*, 2009). Its role in initiating and maintaining pregnancy, mammary gland development and differentiation has made it a potential biomolecule for genetic studies (Nemir *et al.*, 2000). A large number of polymorphisms located in noncoding regions, including promoters, 5' and 3' untranslated gene regions (UTR), introns, and intergenic portion, associated with milk production traits have been identified so far (Yudin and Voevoda, 2015). Osteopontin gene has been reported for 8514C>T (intron IV) and other novel polymorphisms in coding and non-coding regions that were associated with milk production traits and body weight in various breeds (Khatib *et al.*, 2007; Pareek *et al.*, 2008; Mello *et al.*, 2012; Kowalewska-Luczak and Kulig, 2013; Nani *et al.*, 2015; Pasandideh *et al.*, 2015). This work was planned to genetically characterize the osteopontin gene in riverine buffalo and to identify the single nucleotide polymorphism (SNP) as genetic markers.

## Materials and methods

In order to analyze the Osteopontin polymorphism, fifty blood samples of Nili Ravi (Riverine buffalo) were collected from different livestock farms and processed for genomic DNA extraction (Maryam *et al.*, 2012).

Specific primer set 5' -TATAGACTTTGGTGTGGAAG- 3' (forward), 5' -CCCTGCTTTAATGTATCCTT- 3' (reverse) was designed to amplify the 3'UTR of SPP1 gene in riverine buffalo. A total of 25 µL reaction mixture containing 1 µL DNA (50 ng/µL), 2.5 µL PCR buffer (2mM), 2.5 µL dNTPs (25mM), 2.5 µL MgCl<sub>2</sub>, 1 µL of each forward and reverse primers (10 pmol), 0.75 µL Taq DNA polymerase (5U / µL), and 13.75 µL of deionized water was used for PCR amplification. Then the amplicons were precipitated and sequenced by Sanger chain termination method using AB 3130 XL genetic analyzer (Applied Bio systems Inc., Foster City, CA).

Sequences were aligned by using ClustalW and analyzed for genetic variation. p.37421762 (C/A) was found in 3' UTR of Osteopontin gene. Polymorphism was further analyzed for Chi<sup>2</sup> testing, Hardy Weinberg Equilibrium (HWE) and association analysis by One Way ANOVA (Mean±SE) (Tables I, II). SNP was found obeying HWE. Further p.37421762 was analyzed for RNA secondary structure (Supplementary Fig. 1) and there was

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**Table I.- Allelic and genotypic frequencies and HWE of identified polymorphism in 3' UTR of *SPP1* gene.**

SNP ID	Allele frequency		Genotype frequency			HWE P< 0.05
	A	B	AA	AB	BB	
p.37421762 C/A	0.6829	0.3171	0.2861	0.1651	0.5383	0.0945

**Table II.- Association analysis of identified SNP in 3' UTR of *SPP1* gene with milk composition traits.**

Genetic variation	Milk composition	AA (Mean $\pm$ SE)	AB (Mean $\pm$ SE)	BB (Mean $\pm$ SE)	P-value (0.05)
		n=17	n=29	n=33	
p.37421762 C/A	Milk fat	6.16 $\pm$ 0.6361	6.44 $\pm$ 1.5665	6.31 $\pm$ 0.5129	0.620834
	SNF	7.22 $\pm$ 0.3552	7.48 $\pm$ 0.3487	7.22 $\pm$ 0.1447	0.883244
	Lactose	3.28 $\pm$ 1.7022	3.67 $\pm$ 0.8933	3.44 $\pm$ 0.7762	0.727452
	Protein	2.98 $\pm$ 0.3824	3.53 $\pm$ 0.2377	3.33 $\pm$ 0.3552	0.829834
	Ash	0.73 $\pm$ 0.6338	0.76 $\pm$ 0.4751	0.88 $\pm$ 0.2851	0.744613

significant difference in the termination loop of mRNA that might hinder the termination and Poly-A tailing of the mRNA molecule effecting functionality of the gene.

#### Results and Discussion

Conventionally, phenotypic and biochemical markers have been used to identify a potential animal for economically important traits in dairy breeds. Now, the selection efficiency of complex quantitative traits depends on the identification of candidate genes as well as DNA polymorphism in them. Once a reliable correlation between DNA polymorphisms and economically important traits has determined, such markers or polymorphisms may be incorporated in marker-assisted selection (MAS) breeding programs. Thus, the use of validated markers can substantially increase selection efficacy of animals (Singh *et al.*, 2014). Analysis of animal databases has revealed that about 344 quantitative trait loci (QTL) are associated with milk production traits and 71 with mastitis related traits (Ogorevc *et al.*, 2009; Singh *et al.*, 2014). Association between DNA polymorphism and milk production traits have been reported for a number of genes including: including diacylglycerol acyltransferase 1 (DGAT1), fatty acid synthase (FASN), stearoyl-CoA desaturase 1 (SCD1), CSN1S1, ATP-binding cassette, subfamily G, member 2 (ABCG2), growth hormone receptor (GHR), oxidised low-density lipoprotein receptor 1 (OLR1), prolactin (PRL), signal transducer activator of transcription 5A (STAT5A), osteopontin (SPP1), leptin, alpha-lactalbumin (LALBA), Transcription factor Pit 1 (POU1F1), CYP11B1 and PPARGC1A (Kaupe *et al.*, 2007; Khatib *et al.*, 2007; Boleckova *et al.*, 2012; Alim *et al.*, 2013; Singh *et al.*, 2014; Maryam *et al.*, 2015; Yudin and Voevoda, 2015; Nadeem and Maryam, 2016).

The SPP1 gene was selected for association test with milk composition traits as several reported markers has showed its effects on milk production (Leonard *et al.*, 2005). Aligned sequence showed an SNP in 3' UTR of SPP1 gene in riverine buffalo (Table I). DNA mutations in 3' UTR and intronic portion of the genes may alter the mRNA stability and splice sites of pre-mRNA, consequently leading to the assemblage of truncated proteins (Yudin and Voevoda, 2015). The identified SNP 37421762 (C>A) in the 3'UTR of the gene was found non-significant in all analyzed milk production traits. The SPP1 gene has been reported for g.8514C>T polymorphism associated with milk composition traits (Leonard *et al.*, 2005; Khatib *et al.*, 2007). However, the current study was found contradictory to them and no SNP was identified in this region of the gene. Cohen *et al.* (2004) reported that SPP1 is a candidate gene affecting milk protein percentage, but the identified SNP in 3'UTR did not show any significant relationship with milk protein content. It may be due to environmental or epigenetic effect, sampling number or a breed difference. However, the effects of genes used in marker-assisted selection (MAS) program should be known and consistent in different environments and genetic backgrounds to increase the selection efficacy of dairy animals.

#### Supplementary material

There is supplementary material associated with this article. Access the material online at: <http://dx.doi.org/10.17582/journal.pjz/2017.49.4.sc8>

#### Statement of conflict of interest

Authors have declared no conflict of interest.

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