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Research article

Pretentious genomic selection signatures in *CYP19A1* gene associated with silent estrous behavior in water buffalo in Pakistan

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ABSTRACT

Background: Poor reproductive efficiency of river buffalos hampers the production capabilities of animals. Buffalos are mainly considered poor breeders owing to the constrained expression of estrus behavior. Failure to display heat signs is an indication of improper functionality of signaling peptides to trigger on a series of behavioral changes, which can be detectable by breeders for timely insemination of females. This might cause an animal to be a repeat breeder. Genomic variations underlying synthesis of signaling peptides can be a useful marker to select superior animals with better reproductive efficiency. In this context, the current study was designed to analyze the *CYP19A1* gene in Nili-Ravi buffalo.
Results: A total of 97 animals were selected and were divided into two groups on the basis of their heat score. PCR amplification and sequencing of the amplicons were performed using the specific sets of primer, and then, sequences were analyzed for novel variants. A total of 11 polymorphic sites were identified illustrating phenotypic variation in the heat score. Most of the loci were found homologous. Single Nucleotide Polymorphisms (SNPs) were analyzed for association with silent estrus. A three-dimensional protein model was also generated to locate the position of exonic SNPs.
Conclusion: This study illustrated that polymorphic sites in the *CYP19A1* gene provided potential markers for selection of buffalos with better estrus behavior.

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1. Introduction

Buffalos are mainly considered as shy breeders; apart from being shy breeders, they exhibit some major reproductive constraints that result in less exploitation of their reproductive efficiency. Among the major reproductive constrains in buffalos, the expression of silent heat is the major cause of less exploitation of the reproductive efficiency of buffalos.

Silent heat is a condition in which the buffalos do not exhibit the behavioral symptoms of estrus (heat), although the physiological symptoms of estrus are present [1,2,3]. As a result of silent heat, animals become repeat breeders and fail to maintain the estrus regularity and cyclicity [4,5,6].

By studying the expressions of different genes involved in the expression of estrus and their underlying mechanisms, we can have an insight into why silent heat is most commonly observed in buffalos as compared to cows. This would help us in exploiting the reproductive efficiency of buffalos [7,8,9]. To understand the underlying mechanisms and the genes involved in controlling the estrus behavior in buffalos, the current study serves to identify novel polymorphic sites in the *CYP19A1* gene in river buffalos, which illustrated a significant association toward silent heat [10,11].

The *CYP19A1* gene in bovine species is located on chromosome 10 and was mapped to band q2.6 (Fig. 1). The gene has 10 exons, and the gene size ranges from 56 to 120 kb in different species [6,10]. It codes for the aromatase protein, which is 503 amino acids (AAs) in length. The molecular weight is 5,996,238.60 Da (GenBank Accession No. AC_000167.1). *CYP19A1* has been a very crucial responder in many reproductive pathways [5,12,13,14,15]. In this study, the *CYP19A1* gene

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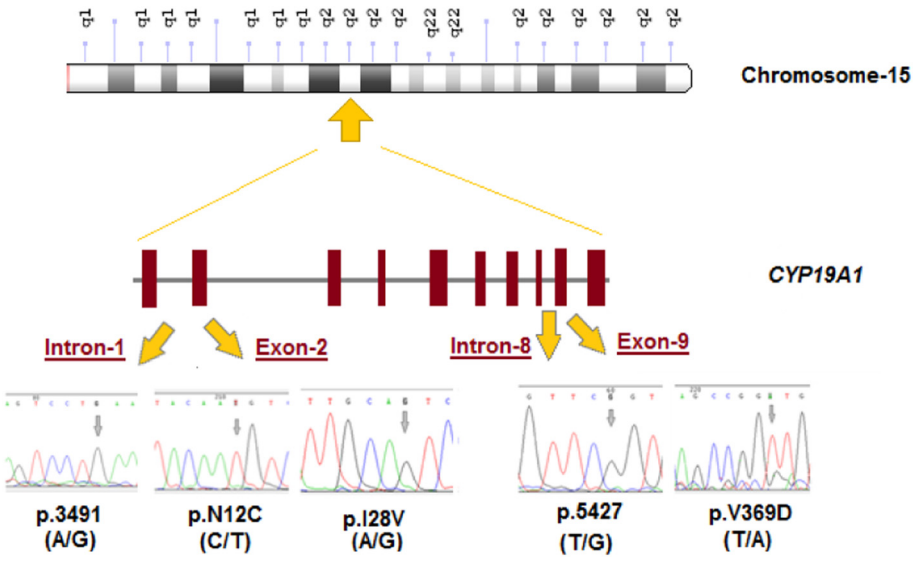

Fig. 1. Structural configuration of the *CYP19A1* gene and the identified polymorphic sites potentially associated with silent heat in Nili-Ravi buffalo.

was characterized by polymerase chain reaction (PCR) amplification and sequencing to identify genomic variants that are associated with silent estrus behavior in bovines.

2. Material and methods

The present study was conducted to identify the single nucleotide polymorphisms (SNPs) in the coding regions of the *CYP19A1* gene that affects the estrus behavior in Nili-Ravi buffalo, resulting in the poor expression of estrus behavior that ultimately leads to the silent heat or estrus condition in buffalos. The research was performed at Molecular and Genomics Laboratory, Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences (UVAS), Lahore.

2.1. Animal selection criteria

A total of 97 genetically related animals that belong to different families, showing typical phenotypic features for a given breed, were selected from several respective breeding tracts with fertility records up to 3 productive cycles from UVAS Pattoki Campus, Research Farm B and Buffalo Research Institute (BRI) Pattoki.

2.2. Sampling strategy

Animals were categorized into two groups on the basis of estrus efficiency. Estrus efficiency was determined by calculating the heat score for each individual female animal by the method reported by Roelofs et al. [15]. In this system, behavioral symptoms (urination, mucus discharge, sniffing, mounting, standing heat, etc.) of estrus after 2 to 3 intervals were recorded, and an individual score was assigned to each sign. Every time when a symptom was observed, a score was assigned to the symptom and was recorded; if the total sum of the score was equal to 50 during two consecutive observations, then the animal was considered to be showing good expression of heat signs, and if the total sum did not exceed 50 then the animal was considered to be showing low signs of heat [15]. In this context, a blood sampling was created on the basis of the most observable heat signs of estrus to obtain the required information, and animals were grouped accordingly.

Buffalos were categorized into two groups, one that showed good signs of heat and the other that showed poor signs of heat, and blood sampling was carried out in both the groups. While selecting the animals for good heat signs, season is a very important parameter. The buffalo expresses good signs of heat during the autumn season. Blood sampling for the current research was carried out during the winter season, i.e., during the months of November and December when there was no seasonal stress on animals and estrus manifestation was at its peak.

2.3. Blood sampling

Blood sampling was conducted after permission from UVAS Ethical Committee (DR/1091). Blood samples (10 ml) from each animal were collected aseptically from the jugular vein into a 50-ml Falcon tube containing 200 μl of anticoagulant, i.e., EDTA (Ethylenediaminetetraacetic acid). After the collection of blood samples, they were placed on ice and were transferred to the Molecular Biology and Genomics Laboratory, IBBT, UVAS Lahore, and were stored at −20°C before DNA extraction.

2.4. DNA extraction and quantification

DNA was extracted from the blood samples using the inorganic method of DNA extraction [12,16]. DNA quantification was done by using NanoDrop and 0.8% agarose gel electrophoresis.

Table 1
Primer sets used to amplify the partial exonic (Exons 2, 3, and 9) region of the *CYP19A1* gene.

Exons	Primer names	Sequences
Exon 2	Forward	GGGCTTGCTTGTTTTGACTC
	Reverse	CTGGTATTGAGGATGTGTCC
Exon 3	Forward	CCCAGCTACTTTCTGGGAAT
	Reverse	CTCAGGTCTCAAGCAAACC
Exon 9	Forward	TCTACGGAACAAGCACAGGA
	Reverse	GGCACGCTCAGTTTTAAGGA