

Identification of Single Nucleotide Polymorphism in 5'-UTR of CYP11B1 Gene in Pakistani Sahiwal Cattle

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Abstract—A major goal in animal genetics is to understand the role of common genetic variants in diseases susceptibility and production traits. Sahiwal cattle can be considered as a global animal genetic resource due to its relatively high milk producing ability, resistance against tropical diseases and heat tolerant. CYP11B1 gene provides instructions for making a mitochondrial enzyme called steroid 11-beta-hydroxylase. It catalyzes the 11deoxy-cortisol to cortisol and 11deoxycorticosterone to corticosterone in cattle. The bovine CYP11B1 gene is positioned on BTA14q12 comprises of eight introns and nine exons and protein is associated with mitochondrial epithelium. The present study was aimed to identify the single-nucleotide polymorphisms in CYP11B1 gene in Sahiwal cattle breed of Pakistan. Four polymorphic sites were identified in exon one of CYP11B1 gene through sequencing approach. Significant finding was the incidence of the C→T polymorphism in 5'-UTR, causing amino acid substitution from alanine to valine (A30V) in Sahiwal cattle breed. That Ala/Val polymorphism may serve as a powerful genetic tool for the development of DNA markers that can be used for the particular traits for different local cattle breeds.

Keywords—CYP11B1, single nucleotide polymorphism, sahiwal cattle, Pakistan.

I. INTRODUCTION

CATTLE (*Bos taurus*) was considered one of the first animal species to come into the genomics era. Identification of Single Nucleotide Polymorphism (SNPs) may be a promising approach to understand and to explain the physiological background of economically important traits. Through the sequenced genome of cattle, more than 2.2 million putative SNPs were recognized [20]. These can be used to investigate kinship [11], individual identification [8], parentage inference [1] and population structure [16].

Steroid 11-beta-hydroxylase (CYP11B1) catalyzes the 11 deoxy-cortisol to cortisol and 11deoxy-corticosterone to corticosterone hormones in cattle [7], [13]. Steroid hormones are physiological regulators and cortisol is one of the principal hormones involved in lipogenesis and lipolysis [4]. The bovine CYP11B1 gene is situated in chromosomal region BTA14q12

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[10] adjacent to marker ILSTS039 [14]. This marker is linked with milk yield as well as with milk component yields [14], [22]. The present research work has been planned to genetically characterize the bovine CYP11B1 gene and to identify single nucleotide polymorphism as genetic markers in local Sahiwal breed.

II. MATERIALS AND METHODS

Unrelated Sahiwal Cattle (unlike families having no blood relation) with typical phenotypic features were selected. Blood samples from representative individuals were collected by 10mL disposable syringes and preserved in 50mL falcon tubes having 400mL Ethylenediamine tetra-acetic acid (0.5M EDTA) as anticoagulant. The sampling was done from different Government livestock farms as Research Centre for Conservation of Sahiwal Cattle (RCCSC) Khanewal and Livestock Production and Research Institute (LPRI) Bahadarnagar Okara.

Genomic DNA was extracted by using standard Phenol Chloroform Isoamylalcohol (PCI) protocols, dissolved in low TE buffer (pH 8.0). The final concentration of all DNA samples was up to 50 ng/μl through Gel electrophoresis (0.8% agarose) and NanoDrop ND-1000 spectrophotometer (Nano Drop Technologies). Primer were designed for *Bos taurus* (Gene Bank Accession no. NC_007312, BTA14, whole genome shotgun sequence) by web based software, "Primer3" (<http://www.primer3.com>). The PCR reaction mixture consist of 2μl DNA (50 ng/μl), 0.75 μl of forward and reverse primers (10 pmol), 2μl PCR buffer (2mM), 2.0 μl dNTPs (25mM), 2.0μl MgCl₂, 0.15μl Taq Polymerase (5U / μl), and deionized water, 14.35μl. All primers were amplified by touchdown PCR protocol with annealing temperature range (62°C-52°C) on Bio-Rad and peQLab thermocycler. The desired amplified portion of DNA was precipitated by 70% ethanol in dark and PCR products were sequenced through ABI prism 3100 genetic analyzer (Applied Biosystems Inc., Foster City, CA). Pairwise alignment of sequence was done with the help of blast2 sequence.

III. RESULTS AND DISCUSSION

Bovine chromosome 14 has extensively studied for quantitative trait loci (QTL) related to economically major traits of dairy and beef cattle [15], [22]. In dairy cattle, the majority of mapped QTL on BTA14 are considered to linked with milk production traits as milk yield, fat percentage (%)

fat yield, protein content (%) and protein yield [2], [3], [5], [9], [14], [17]-[19], [21]. The bovine CYP11B1 gene is positioned in chromosomal region BTA14q12 [10] near marker ILSTS039. This marker is associated with milk yield as well as with milk component yields [22]. Many milk

production genes have been identified so far and work is reported on DGAT1 [3], [9], PRL [6], PPARGC1A [6], [12], CYP11B1 [6], [9] in bovine milk. Keeping in view the above facts a research plan was made to conduct a study on genetic characterization of CYP11B1 gene in local Sahiwal breed.

TABLE I
ALLELE FREQUENCIES IN SAHIWAL CATTLE

Serial No.	SNP ID	ChromosomalPosition	Reference Nucleotide	Changed Nucleotide	Allele Frequency		Major Allele Frequency
1	CYP1	1310397	A	G	A 0.3415	G 0.6585	0.3415
2	CYP2	1310450	G	A	A 0.6585	G 0.3415	0.3415
3	CYP3	1310462	G	A	A 0.6707	G 0.3293	0.3293
4	CYP4	1310487	G	A	A 0.6220	G 0.3780	0.3780
5	CYP5	1310519	A	G	A 0.3659	G 0.6341	0.3659

Five polymorphic sites were identified by using BLAST in local Sahiwal breed. Data reveals that the chromosomal loci 1310397, 1310450, 1310462, 1310487, 1310519 lying in exon one has nucleotide change in the order of A>G, G>A, G>A, G>A and A>G. The nucleotide substitution at P1310487 revealed the Alanine (A) to Valine (V) polymorphism (V30A) in CYP11B1 gene product (Fig. 2). Similar finding was reported [9] that there was a polymorphism (V30A) in 5' UTR and exon one of bovine CYP11B1 gene. They had executed an association test between bovine CYP11B1 gene and milk production traits in German Holstein and the results showed the genetic variability in 5' UTR and exon one is highly associated with milk production traits. Another association study also stated significant effect of the Ala/Val polymorphism in bovine CYP11B1 gene in Czech Fleckvieh cows [7]. A more recent study was implemented on CYP11B1 gene and results revealed the p.Val30Ala polymorphism in the first exon of gene [6]. Accordingly the CYP11B1 polymorphism had shown positive associations with milk composition traits and breeding values for milk yield, fat contents and composition of protein in different cattle breeds.

A relationship was established between the amino acids composition of reference versus subject protein (Fig. 2) by using BioEdit translate tool. Though the majority of the reported SNP do not bring amino acid change, these sites may be related to detect causative mutation or adjacent QTL. Results also showed the distribution pattern of alleles and their frequencies against each recognized SNP in Sahiwal local breed (Table I). However the identified polymorphic sites were considered breed specific and might be correlated to milk production traits.

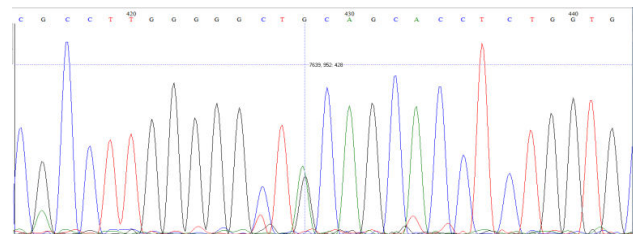


Fig. 1 SNP polymorphism (G→A at Position no. 1310487) shown in sequencing results of CYP11B1 gene in Sahiwal cattle

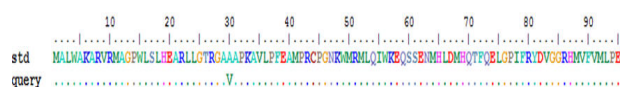


Fig. 2 Ala/Val polymorphism in CYP11B1 gene product in Sahiwal cattle

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