World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:7, No:10, 2013

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

S. Manzoor, A. Nadeem, M. Javed, ME. Babar

***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 corticosteronehormones 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 [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.

S. Manzoor is with the Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences Lahore 54000, Pakistan (phone: +92-321-6726428; e-mail: sidzm@yahoo.com).

M. Javed and A. Nadeem are with Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences Lahore 54000, Pakistan (e-mail: maryam.javed@uvas.edu.pk, asif_cemb@hotmail.com).

ME Babar is Faculty of Animal Production & Technology, University of Veterinary and Animal Sciences Lahore 54000, Pakistan (e-mail: mbabar@uvas.edu.pk).

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 donefrom 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 Technolgies). 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 $MgCl_2$, 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.

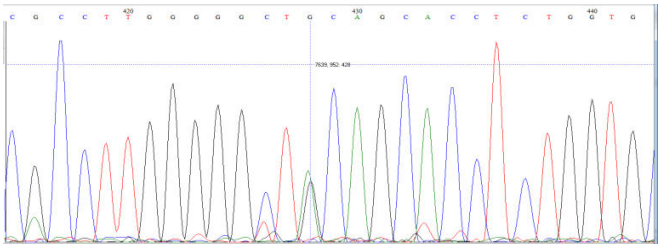

Fig. 1SNP polymorphism (G→A at Position no. 1310487) shown in sequencing results of CYP11B1gene in Sahiwal cattle

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

REFERENCES

[1] Anderson EC, Garza JC. 2006. The power of single-nucleotide polymorphisms for large-scale parentage inference. Genetics 172: 2567–2582.
[2] Bagnato A, Schiavini F, Rossoni A. 2008. Quantitative trait loci affecting milk yield and protein percentage in a three-country Brown Swiss population. J Dairy Sci. 91:767-783.
[3] Bennewitz, J, Reinsch N, Paul S, Looft C, Kaupe B, Weimann C, Erhardt G, Thaller G, Kuhn C, Schwerin M, Thomsen H, Reinhardt H, Reents R, Kalm E. 2004. The DGAT1 K232A mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. J Dairy Sci. 87:431–442.
[4] Bhathena, SJ. 2000. Relationship between fatty acids and the endocrine system. Biofactors 13:35–39.
[5] Boichard D, Grohs C, Bourgeois F. 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. Genet SelEvol. 35:77-101.
[6] Boleckova J, Matejickova J. Stipkova M, Sefrova J, Krejcova M, Barton L. 2012. The association of five polymorphisms with milkproduction traits in Czech Fleckvieh cattle. J Anim Sci. 57 (2): 45–53.
[7] Boleckova J, Matejickova J. Stipkova M, Sefrova J, Krejcova M. 2010. Polymorphism OfCYP11B1 Gene And Its Relation To Milk Production Traits In Czech Fleckvieh Cattle. Institute of Animal Science, Pratelstvi 815, 104 00, Prague Uhrineves, Czech Republic.

[8] Chakraborty R, Stivers DN, Su B, Zhong Y, Budowle B. 1999. The utility of short tandem repeat loci beyond human identification: Implications for development of new DNA typing systems. Electrophoresis 20: 1682–1696.
[9] Kaupe B, Brandt H, Prinzenberg E-M, Erhardt G. 2007. Joint analysis of the influence of CYP11B1 and DGAT1 genetic variation on milk production, somatic cell score, conformation, reproduction, and productive lifespan in German Holstein cattle. J Anim Sci. 85:11-21.
[10] Kaupe, B., S. Kollers, R. Fries, and G. Erhardt. 2004a. Mapping of CYP11B1 and a putative CYHR1 paralogous gene to bovine chromosome 14 by FISH. Anim. Genet. 35:6.
[11] Krawczak M. 1999. Informativity assessment for biallelic single nucleotide polymorphisms. Electrophoresis 20: 1676–1681.
[12] Li H, Wang Z, Moore SS, Schenkel FS, Stothard P. 2010. Genome-wide Scan For Positional And Functional Candidate Genes Affecting Milk Production Traits In Canadian Holstein Cattle. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, T6G2P5.
[13] Lisurek, M., Bernhardt R. 2004. Modulation of aldosterone and cortisol synthesis on the molecular level. Mol Cell Endocrinol. 27:149–159.
[14] Looft C, Reinsch N, Karall-Albrecht C. 2001. A mammary gland EST showing linkage disequilibrium to a milk production QTL on bovine Chromosome 14. Mamm Genome. 12:646-650.
[15] Marques E, de-Givry S, Stothard P. 2007. A high resolution radiation hybrid map of bovine chromosome 14 identifies scaffold rearrangement in the latest bovine assembly. BMC Genomics. 8:254.
[16] Morin PA, Martien KK, Taylor BL. 2009. Assessing statistical power of SNPs for population structure and conservation studies. Molecular Ecology Resources 9: 66–73.
[17] Rodriguez-Zas SL, Southey BR, Heyen DW, Lewin HA. 2002. Interval and composite interval mapping of somatic cell score, yield, and components of milk in dairy cattle. J Dairy Sci. 85:3081-91.
[18] Schnabel RD, Sonstegard TS, Taylor JF, Ashwell MS. 2005. Whole-genome scan to detect QTL for milk production, conformation, fertility and functional traits in two US Holstein families. Anim Genet. 36:408-416.
[19] Thaller G, Krämer W, Winter A, Kaupe B, Erhardt G, Fries R. 2003. Effects of DGAT1 variants on milk production traits in German cattle breeds. J Anim Sci. 81:1911-1918.
[20] Via S, West J. 2008. The genetic mosaic suggests a new role for hitchhiking in ecological speciation. Molecular Ecology 17: 4334–4345.
[21] Viitala SM, Schulman NF, de Koning DJ, Elo K, Kinos R, Virta A, Virta J, Mäki-Tanila A, Vilkki JH. 2003. Quantitative trait loci affecting milk production traits in Finnish Ayrshire dairy cattle. J Dairy Sci. 86:1828-1836.
[22] Wibowo TA, Gaskins CT, Newberry RC, Thorgaard GH, Michal JJ, Jiang Z. 2008. Genome Assembly Anchored QTL Map of Bovine Chromosome 14. Int J Biol Sci. 4(6):406-414.