POLYMORPHISM IN STEROID 11-BETA-HYDROXYLASE GENE EFFECTS ON MILK FAT CONTENT IN SAHIWAL CATTLE

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ABSTRACT

Genetic improvements of livestock is mainly depends on selective breeding of animal with its superior phenotypes. Identification and use of molecular markers for production and economical traits has been ensured the better health and productivity of cattle. Cytochrome P450, family 11, subfamily b, polypeptide 1 (*CYP11B1*) gene is a candidate affecting milk composition traits as adjacently located to marker ILSTS039 that has been linked with milk yield and milk component. The current study was performed to characterize the CYP11B1 gene in Sahiwal cattle. The overall sequence variation across the *CYP11B1* locus is high and total thirteen variations were observed in Sahiwal. Association test was executed against all variations obeying Hardy Weinberg Equilibrium (HWE) and found non-significant except g.1305935 (T401M) that was found associated with milk fat percentage in Sahiwal population. It is illustrated that *CYP11B1* gene has an influential dairy potentials in cattle can also be incorporated into genetic evaluation.

Keywords: *CYP11B1*, SNPs, Association, Milk fat percentage, Sahiwal cattle.

INTRODUCTION

Identification of Single Nucleotide Polymorphism (SNPs) is a promising approach to understand and explain the physiological background of economically important traits. More than 2.2 million putative SNPs were recognized (Via and West, 2008) and used to investigate kinship (Krawczak, 1999), individual identity (Chakrabort *et al*., 1999), paternity implication (Anderson and Garza, 2006) and population structure (Morin *et al*., 2009). Cattle were considered one of the first animal species to come into the genomics era. Pakistan is known as the fifth largest producer of milk in the world. The national herd consists of 42.8 millions of cattle, producing 19,412 million tons of milk (Economic survey of Pakistan 2015-16). Among various indigenous breeds, Sahiwal and Red Sindhi are internationally well-known tropical dairy breeds (Khan *et al*. 2008). In present study the attention was paid on Sahiwal breed that is the best milking breed of the country. It named after a tribenamed "Saho" that lived in Sahiwal district. Milk yield is 1,550 litres per lactation (Rehman, 2006) with 4.5 % fat content. It is tick resistant and heat tolerant.

Steroid 11β-hydroxylase, encoded by the *CYP11B1* gene, belongs to a class of enzymes involved in the biosynthesis of steroid hormones (cortisol and corticosterone), milk production, energy metabolism, somatic cell score, and reproduction (Kaupe *et al*., 2007). The bovine *CYP11B1* gene has been mapped on chromosomal region BTA14q12, comprises of nine exons and eight introns and nucleotide stretch of its reference sequence is about 7.679 kbp (http://www.ncbi.nlm.nih.gov/gene/282422). It has been reported that bovine *CYP11B1* gene is closely located to marker ILSTS039, linked with milk yield and milk component (Wibowo *et al*., 2008). This *CYP11B1* has been reported as a functional and positional DNA marker associated to various production traits. Thus, the current work was planned to identify the single nucleotide polymorphisms in *CYP11B1* gene and their relation with milk fat percentage in local Sahiwal breed.

MATERIALS AND METHODS

Sampling Strategies: Animals of Sahiwal breed in second lactation were selected to collect the blood and milk samples. Milk samples were collected for fat percentage analysis thus animals were categorized into two groups on the basis of milk fat percentage. Animals (n = 25) with more than 4 percent of fat were included in group A while animals (n = 25) with less than 4 percent of fat were included in group B. The sampling was done from Government livestock farms Research Centre for Conservation of Sahiwal Cattle (RCCSC) Khanewal.

DNA Amplification and Sequencing: The genomic DNA of all samples was extracted by using the standard Phenol Chloroform Isoamylalcohol (PCI) protocols, dissolved in low TE buffer (pH 8.0). The final concentration of all DNA samples was upto 50 ng/µl through Gel electrophoresis (0.8 % agarose) and

NanoDrop ND-1000 spectrophotometer (NanoDrop Technolgies). Eight sets of 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 2 µl DNA (50 ng/µl),0.75 µl of forward and reverse primers(10 pmol), , 2.0 µl PCR buffer (2mM), 2.0 µl dNTPs (25mM),2.0 µl$MgCl_2$, 0.15 µl 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 peQ Lab 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). Sequences were aligned using BLAST and a total of thirteen polymorphisms were identified in CYP11B1 gene.

Statistical Analysis: Allelic and genotypic frequencies for the observed SNPs were calculated using Bioinformatics software POPGENE version 1.32 (http://www.ualberta.ca/fyeh/) and Hardy Weinberg Equilibrium (HWE) was also examined by using Chi2 test. Association analysis was performed by using one way ANOVA (Mean±SE).

RESULTS AND DISCUSSION

Recent development in animal genetics enables us to measure polymorphic loci associated with animal health and production traits. There has been strong focus on single genes and mapping QTL to make them available in near future (Singh *et al*., 2014). Bovine chromosome 14 has extensively studied for quantitative trait loci (QTL) related to economically major traits of cattle (Wibowo *et al*., 2008). In dairy cattle, the majority of mapped QTL on BTA14 are considered to linked with milk production traits as milk yield, fat content (%), fat yield, protein percentage (%) and protein yield (Bennewitz *et al*., 2004; Boichard *et al*., 2003; Kaupe *et al*., 2007). The bovine *CYP11B1* gene is nearly positioned to marker ILSTS039. This marker is associated with milk components and yield (Wibowo *et al*., 2008). The *CYP11B1* also influence the cortisol production, function of androgen and ultimately the proliferation of milk gland cells (Javed *et al*., 2013). Cortisol remains a principal hormone involved in lipogenesis and lipolysis. This makes the *CYP11B1* locus of particular interest and a key candidate for association studies. Keeping in view the above facts a research work was done to find the probable markers in *CYP11B1* gene and their association with milk production traits in local Sahiwal breed.

The overall sequence variation across the bovine *CYP11B1* locus is high and thirteen novel polymorphisms were identified (Table I). Among them, seven substitutions were laid in coding region of the gene while six were found in intronic (near to exonic) portion of the gene. The distribution pattern of alleles and genomic frequencies against each recognized SNP (Table 2) and HWE were determined. SNPs obeying HWE were considered for association analysis. Significant finding of the study was the incidence of two adjacent nucleotides change (1306512 and 1306513) in exon six, constituting a single codon transcribed into threonine to Aspartic acid (Thr372Asp). Threonine is hydroxyl containing amino acid and is polar neutral in nature while aspartic acid is a polar negative; acidic in nature, is a clear indication that the nature of final protein product of the gene will be changed. Exon eight has also two nucleotide changes at 1305894 and 1305935 positions, responsible for the Ala415Thr and Thr401Met substitution respectively. The *CYP11B1* gene has been associted with milk production, somatic cell score, energy metabolism and reproduction in various breeds. We also performed an association test of all identified polymorphisms with milk fat percentage. All substitutions were found non-significant except g.1305935 that was found associated with milk fat percentage in Sahiwal population (Table 3). Kaupe *et al*., (2007) had also executed an association test between *CYP11B1* gene and milk production traits and reported a highly associated polymorphism (V30A) in 5 UTR and exon one of the gene in German Holstein. Boleckova *et al*., (2012) also stated significant effect of the p.Val30Ala polymorphism in Czech Fleckvieh cows. We have previously reported V30A polymorphism in Sahiwal (Manzoor *et al*., 2013) but it was not analyzed for association study. However, that SNP was also structured the local breed population. In another study, a novel polymorphism A313T has been associated with milk fat percentage in riverine buffalo (Maryam *et al*., 2015). All other polymorphic sites were present in non-coding portion of the gene that does not bring amino acid change; these sites may be related to detect causative mutation or adjacent QTL. However the identified polymorphic sites were considered breed specific.

Table 1. Identified Polymorphisms in bovine *CYP11B1*gene with HWE (p<0.05).

Sr. No.	SNP ID	Chromosomal Position	Change in Nucleotide	Intronic/ Exonic	Transition/ Transversion	Change in Codon	Amino acid Change	HWE (P<0.05)
1	CYP1	1305769	A→G	Intronic	Transition	--	--	0.009027*
2	CYP2	1305894	G→A	Exonic	Transition	GCC > ACC	Alanine → Threonine	0.03523*
3	CYP3	1305935	C→T	Exonic	Transversion	ACG > ATG	Threonine→Methionine	0.447982**
4	CYP4	1306512	C→A	Exonic	Transversion	ACC > GAC	Threonine → Aspartic acid	0.0014567*
5	CYP5	1306513	A→G	Exonic	Transversion	ACC > GAC	Threonine → Aspartic acid	0.013462*
6	CYP6	1306604	C→T	Exonic	Transversion	CGC > CGT	Arginine → Arginine	0.0000081*
7	CYP7	1306609	G→C	Exonic	Transversion	GTG > CTG	Valine → Leucine	0.000021*
8	CYP8	1306610	G→C	Exonic	Transversion	GCG > GCC	Alanine → Alanine	0.05627*
9	CYP9	1308462	A→G	Intronic	Transition	--	--	0.061367*
10	CYP10	1308463	G→A	Intronic	Transition	--	--	0.13479**
11	CYP11	1308490	G→T	Intronic	Transversion	--	--	0.207161**
12	CYP12	1308721	G→C	Intronic	Transversion	--	--	0.034681*
13	CYP13	1308723	T→G	Intronic	Transversion	--	--	0.030567*

*Significant
**Non-Significant

Table 2. Allelic and Genotypic Frequency of identified polymorphisms.

Sr.No.	SNP ID	Allele Frequency		Genotype Frequency		
1	CYP1	A	G	GG	AG	AA
		0.6041	0.3959	0.5484	0.0645	0.3871
2	CYP2	G	A	GG	AG	AA
		0.3293	0.6707	0.2683	0.1220	0.6098
3	CYP3	C	T	CC	CT	TT
		0.2927	0.7073	0.2195	0.1463	0.6341
4	CYP4	C	A	CC	CA	AA
		0.2927	0.7073	0.2439	0.0976	0.6585
5	CYP5	A	G	AA	GA	GG
		0.2805	0.7195	0.2195	0.1220	0.6585
6	CYP6	C	T	CC	CT	TT
		0.6829	0.3171	0.5854	0.1951	0.2195
7	CYP7	G	C	CC	CG	GG
		0.3537	0.6463	0.2683	0.1707	0.5610
8	CYP8	G	C	GG	CG	CC
		0.3659	0.6341	0.2683	0.1951	0.5366
9	CYP9	A	G	GG	AG	AA
		0.6829	0.3171	0.5484	0.0645	0.3871
10	CYP10	G	A	AA	AG	GG
		0.6029	0.3971	0.5806	0.0645	0.3548
11	CYP11	G	T	TT	GT	GG
		0.7707	0.2293	0.4516	0.0968	0.4516
12	CYP12	G	C	GG	CG	CC
		0.3293	0.6707	0.3871	0.0323	0.5806
13	CYP13	T	G	TT	GT	GG
		0.6063	0.3937	0.4516	0.0968	0.4516

Table 3. Association Analysis of polymorphic sites obeying HWE using One Way ANOVA.

SNP ID	AA	AB	BB	P-value
CYP3	2.91±0.6869	3.41±0.4934	4.14±0.2465	0.003813
CYP10	3.12±0.6207	3.24±0.7521	3.22±0.7521	0.41095
CYP11	3.22±1.529	3.01±0.6077	3.12±0.2695	0.124121

Conclusion: Significant polymorphisms that bring amino acids change include the V340L, T372D, T401M and A415T in *CYP11B1* gene. Polymorphism g.1305935 (T401M) was found associated with milk fat percentage. Identified polymorphism can be used as probable marker for more production and economical traits. Besides the identification of SNPs, gene expression patterns dependent review will help us to understand the molecular mechanisms of this gene and its role in production traits of dairy animals. Therefore, functional validation of polymorphisms in *CYP11B1* gene should be performed in the future.

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