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Exploring polymorphism at STR ETH10 and its association with birth weight in Vrindavani cows

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Abstract
Short tandem repeats have served as markers to locate loci controlling a quantitative trait. The present study was undertaken to identify genetic variants at the short tandem repeat region ETH10 in Vrindavani cows to determine their association with body weight at birth (BW) and thus to locate a segregating QTL for this quantitative trait in the population studied. 95 animals of the breed were genotyped for the STR and phenotyped for birth weight. The locus was found to be polymorphic with a total of 3 alleles present in the population. Least square analysis of variance did not reveal any significant effect of genotypes at ETH10 on body weight at birth in Vrindavani cattle.

Keywords: STR, polymorphism, QTL, birth weight, cattle

Introduction
Short tandem repeats (STR) have been utilised to map the genome across species for a very long time.They are DNA motifs that often occur frequently in the genome and consist of 2–6 base pairs repeated 5–50 times, such as (GT)n or (AGCTA)n [7, 4]. These tandem repeats are also known as microsatellites [7], simple sequence repeats (SSR) [14], or simple sequence length polymorphism (SSLP). STRs have been found in the genomes coding and non-coding regions, and they have been used to find QTLs [12]. The STAT6 gene promoter region on bovine chromosome 5 contains a GT repeat called ETH10, a microsatellite that is part of the International Society of Animal Genetics (ISAG) parentage panel. In animals, the signalling of GH, which controls postnatal bone and muscle growth and fat metabolism, involves STAT6 [16].

The genotypes of ETH10 have helped researchers identify QTL and carry out genotype-to-phenotype association studies for cattle growth and carcass traits [5, 13]. Understanding the relationships between genotype and phenotype with the help of polymorphism observed at STR markers offers a chance to learn about QTL and the underlying genes and alleles. These analyses may be complicated by the genetic variability that exists within breeds [8, 10, 15].

Vrindavani is a synthetic cattle breed developed at ICAR-Indian Veterinary Research Institute, Bareilly (India) as an improved dairy breed having inheritance from Holstein, Jersey, Brown Swiss and Hariana. Many studies to genetically improve its production performance have been conducted but the traits of functional importance have not been studied in the breed. Therefore the present study was undertaken to detect an association between a STR locus ETH10, whose linkage with QTL controlling growth traits has already been reported, and functionally important trait of body weight at birth.

Material and Methods
Experimental animals and data collection
A total of 95 Vrindavani cows maintained at the institutional herd were included in the study. Data on the body weight of each cow on the day of birth was collected from the records available at the farm data unit. Only the animals born from normal parturition were included in the study.

Sample collection and DNA isolation
About 5 ml blood was collected from the jugular vein of each animal EDTA vaccutainers and stored at -20 ºC. DNA was isolated from stored samples post thawing using Phenol chloroform DNA extraction method given by Sambrook and Russel (2001) [11].

Genotyping of ETH10

The sequence of ETH10 was downloaded from NCBI and primers were designed using Primer3 software. For PCR amplification of the STR a reaction mixture was prepared in a 200 µl nuclease free PCR tube to a final volume of 25 µl by adding the following components: PCR Master Mix (2X)- 12.5 µl, Forward Primer (10 pmol/µl)- 1 µl, Reverse Primer (10 pmol/µl)- 1 µl, Genomic dna - 2 µl, Nuclease free water- 8 µl. Amplification was carried out in athermal cycler after the thermal conditions have been optimized with annealing at 58 ºC.

PCR products from each Vrindavani cow were resolved for the alleles of ETH10 by MetaPhor Agarose Gel Electrophoresis (MAGE). 10µl PCR product was carefully loaded in each well followed by electrophoresis at 100 volts for 3.5 hours. The metaphor agarose gel was visualized in a Gel-doc system (Syngene, USA; and Bio-Rad, U.S.A.) and ETH10 allelic patterns were recorded by photography for genotyping. Gene Tools software (Syngene, USA) of the Gel Doc system was used to estimste the molecular size (in bp) of observed alleles of ETH10 locus. Genotype of each animal was determined on the basis of STR alleles it contained.

Statistical analysis

Association of observed genotypes with birth weight was determined by PROC GLM procedure of SAS 9.3 software. The model used was

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where,

Y_{ij}= j^{th} observation on birth weight for i^{th} genotype

μ = over all mean,

G_i= effect of i^{th} genotype, and

e_{ij}= error term

Results

The number, frequency and size of the alleles and the genotypes observed at ETH10 have been presented in tables 1 and 2. The STR on MetaPhor Agarose Gel Electrophoresis revealed 3 alleles (A, B and C, which ranged in size from 216 to 238 bp) and 6 genotypes (AA, AB, AC, BB, BC and CC). Of the genotypes observed 3 were homozygotes and the other 3 were heterozygotes. The B allele had the highest frequency (59.47%), while the C allele had the lowest frequency (10.53%).

Table 1: Size, number and frequency of alleles at ETH10

SI. No.	Allele	Allele Size (bp)	Number (n)	Alleleic Frequency (%)
1	A	238	57	30
2	B	224	113	59.47
3	C	216	20	10.53
	Total		190	

Table 2: Genotypes, numbers and their frequency at ETH10

SI. no.	Genotype	Number (n)	Genotype frequency (%)
1	AA	9	9.47
2	AB	26	27.37
3	AC	13	13.68
4	BB	41	43.16
5	BC	5	5.26
6	CC	1	1.05
	Total	95	

Least square analysis of variance did not reveal any significant association of genotypes at ETH10 with birth weight in Vrindavani cattle. The least square mean for birth weight was 22.9 ± 0.4 (95) kg. The genotype AA had the highest mean birth weight of 25.4 ± 1.1 (9) kg whereas genotype BC had the lowest mean birth weight of 21.2 ± 2.7 (5) kg. The least square means (± standard error) of the six genotypes observed at this STR for birth weight have been presented in the Table 3.

Table 3: Least square means with standard error and number of observations for birth weight with respect to ETH10 genotypes

SI. No	Genotype	Birth Weight
1.	AA	25.4 ± 1.1 (9)
2.	AB	23.2 ± 0.8 (26)
3.	AC	21.8 ± 0.9 (13)
4.	BB	22.7 ± 0.7 (41)
5.	BC	21.2 ± 2.7 (5)
6.	CC	22 ± 0.00 (1)
	Total	22.9 ± 0.4(95)

Discussion

The STR ETH10 exhibited polymorphism in the studied Vrindavani population. A total of 3 alleles were seen in Vrindavani cows with the largest allele of the size 238 bp and the smallest allele of the size 216 bp. In a study on Angus and Brahman influenced cattle DeAtley *et al.* (2011) [3] observed a maximum of 13 alleles ranging in size from 199 to 225 bp. The study was conducted on a larger population comprising of several genetic groups. In another study on Bali cattle Margawati *et al.* (2019) [9] obtained 5 alleles from 209 to 219 bp.

The STR ETH10 is present on *Bos taurus* autosome 5 on which several QTLs influencing birth weight have been reportedin different breeds [1, 2, 6]. However, we could not detect any significant association of the genotypes observed at ETH10 on birth weight in Vrindavani cattle. Kim *et al*. (2003) [5] revealed a significant effect of ETH10 on birth weight in Brahman and Angus cattle as well as their crosses. Association of growth traits in Brahman cattle with genotypes at ETH10 was also studied by DeAtley *et al*. (2011) [3] who grouped the different alleles observed at this locus into two groups, large and small. The birth weight of individuals with small/large genotype was significantly higher than that of large/large genotype (p<0.01).

Conclusion

The present study was conducted with the aim to determine polymorphism at ETH10 locus in Vrindavani cattle and to detect the association of observed variants with birth weight. The effect of genotypes at the STR was found to be insignificant in the population. Further studies with a different set of STRs and a larger population should be undertaken to identify segregating QTLs for birth weight in this breed.

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Conflict of interest

The authors declare no conflict of interest.

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