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Allelic diversity of butyrophilin (*BTN1A1*) gene in Indian bovines

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ABSTRACT Indian milch bovines comprise 58.56% of the total livestock population (512.05 million) in the country and primarily include native and crossbred cattle (37.28%) and water buffaloes (21.28%). Milk and milk products are essential food items in Indians' diet, especially in children and the elderly and senile. Milk fat is an important constituent of milk and has an economic value and its percentage in milk varies between species and breeds within species. Butyrophilin (*BTN1A1*) is a membrane protein that regulates the secretion of lipids and size of a fat globule in milk. The present study was conducted on 538 bovines from 11 breeds/populations adapted to different parts of India, with an aim to screen and determine the major allele of the *BTN1A1* gene. The genotyping of samples was done using PCR-RFLP based tests. The results indicated that exon8 of the *BTN1A1* gene was polymorphic in the Tharparkar, Sahiwal, Jhari, and Belahi populations of the native cattle, as well as the Holstein Friesian and Jersey crossbreeds. Meanwhile, exon 8 was monomorphic in the Murrah, Chilika, Gojri, Chhattisgarhi, and Bargur populations of water buffalo. Allele **A** was identified as the major allele in Indian bovines. We conclude that variations in the *BTN1A1* gene can serve as an excellent genetic marker in the selection of cattle with higher milk fat, and can be applied when formulating their breeding plans.

KEYWORDS butyrophilin; fat; milk; polymorphism; SNP

1. Introduction

Indian dairy industry is focused on invention of efficient and economical ways of increasing milk production. Selection of superior milch bovines without increasing the size of dairy herd and further their mating to raise improved progenies is one of the ways to enhance the milk production. Of late, there has been an emphasis to select genetically superior milch bovines based on polymorphic candidate genes and DNA markers rather than conventional phenotypic selection. Number of SNPs has shown positive and significant associations with milk fat secretion among different exotic breeds of cattle but they have not been explored fully among Indian bovines. In India, current selection and breeding strategies focus only to increase milk yield in milch animals. There is need to put efforts to select milch bovines based on milk composition and its quality like fat yield owing to dependency of dairy economics to a large extent on fat yield. Moreover, in organized sector and dairy co-operatives, producers are paid on the basis of fat corrected milk (FCM). Marker based selection followed with suitable breeding methods for high milk fat yield without affecting milk production can help in genetic selection among milch bovines. Selection for milk and milk constituent (fat) traits can be improved by utilizing information from SNPs linked to QTL. It is important to explore genetic variations at DNA level in dairy animals and subsequently their use in formulating breeding programmes. Several studies in exotic cattle have revealed large genetic variations in bovine milk fat and milk fat composition (Soyeurt et al. 2007; Stoop et al. 2008). A number of SNPs in candidate genes having role in fat synthesis are found to be associated with milk fat percentage or milk fat yield. Various candidate genes have been identified for fat synthesis such as Fatty acid synthase (FASN) (Vohra et al. 2015; Kumar et al. 2016b, 2017), Diacylglycerol-acyl-transferase 1 (DGAT1) (Grisart et al. 2002) and Signal transducer and activator of transcription 1 (STAT1) gene (Cobanoglu et al. 2006; Kumar et al. 2015, 2016a). The main aim of genetic research in farm animals is identification of genes influencing economically important traits that could be useful in breeding programme (Asadollahpour Nanaei et al. 2013).

Butyrophilin (*BTN1A1*) is a candidate gene important to milk yield and composition (fat). This protein is directly involved in the secretion of fat globules at the apical surface of mammary epithelial cells throughout lactation (Jack and Mather 1990). DNA level variations may help to identify possible hybridization as well as past evolutionary events and contributes to the genetic characterization

of a livestock population. A protein's expression may be altered by a change in amino acids due to exonic region variations in a gene (Geldermann 1975). A group of membranous proteins helps fat droplets in getting proper shape during secretion and *BTN1A1* plays a significant role during the process of lactogenesis (Bhattacharya et al. 2007). *BTN1A1* is the most abundant protein in milk fat globule membrane and is particularly expressed in lactating mammary tissue. *BTN1A1* is produced at the end of pregnancy and is maintained throughout lactation (Ogg et al. 2004). Among the total protein associated with fat globule membrane of bovine milk, *BTN1A1* constitutes more than 40% by weight (Mather and Jack 1993). This protein is usually sandwiched between plasma membrane and surface of fat droplets (Wooding and Kemp 1975), and its characteristic feature is non-solubility in non-ionic detergent due to its hydrophobic property (Freudenstein et al. 1979). During budding and secretion of fat droplets into milk, *BTN1A1* is incorporated into the milk-fat-globule membrane. The budding of droplets at cell surface is initiated by interactions between cytoplasmic tail of BTN and other proteins like xanthine oxidase, fatty acid synthetase, GTP-binding proteins and lipids, though, butyrophilin may function as an imperative receptor for cytoplasmic fat droplets (Jack and Mather 1990). The structural relationship of butyrophilin to proteins of the immune system is suggestive of its possible immunologic function, distinctive to milk-fat secretion (Taylor et al. 1996a). A number of mammalian species including humans (Taylor et al. 1996b), cattle (Jack and Mather 1990) and mice (Ishii et al. 1995) possess the *BTN1A1* gene. Bovine butyrophilin gene consists of 8 exons and 7 introns and is located on the 23rd chromosome (Ashwell et al. 1996). *BTN1A1* is possibly QTL candidate gene affecting milk yield and composition in dairy animals (Komisarek and Dorynek 2003). Franke et al. (1981) suggested that butyrophilin is widely expressed during lactation and is specific to mammary tissue.

Genetic variations can be analyzed at both the phenotypic and genetic levels and are key factors for the improvement of the performance of animals. It is well-known that every trait is controlled by gene(s) and nowadays it is of utmost importance to identify gene(s) in order to explore genotype–trait relationships. As such, identification of SNPs subsisting in the genome is required to detect the effect of genotypes on economically important traits. SNPs help to know the evolutionary relationships between species along with searching molecular markers. Keeping all this in view, the present study was undertaken to screen variations in the *BTN1A1* gene of different indigenous, crossbred cattle and riverine buffaloes of India.

2. Materials and methods

2.1. Population studied and sample size

The studied bovine populations, namely Murrah (North India), Sahiwal and Tharparker (Northwestern India) and crossbreeds of Holstein Friesian (North India), and Jersey cattle (coastal parts of India) are primary milch breeds and have larger spread across India, contributing significantly to the national milk production. Populations like Belahi and Gojri (North India), Chhattisgarhi and Jhari (Central India), Chilika (Eastern India), and Bargur (South India) besides milk production are also used for draught power and have better adaptability, extensively managed and limited distribution but strong socio-economic utility to their keepers. Random blood samples (approximately 8 to 10 mL) were aseptically collected from the jugular veins of 538 genetically unrelated bovines, specifically Murrah buffalo (n = 200), Gojri buffalo (n = 40), Chhattisgarhi buffalo (n = 40), Chilika buffalo (n = 30), Bargur buffalo (n=30), Tharparkar cow (n = 30), Sahiwal cow (n = 30), Belahi cow (n = 48), Jhari cow (n = 30), Holstein Fries crossbreed cattle (Karan Fries) (n = 30), and Jersey crossbreed cow (n = 30). Samples of Tharparkar, Sahiwal, Karan Fries cattle, and Murrah buffaloes were collected from organized herd of ICAR-National Dairy Research Institute, Karnal (Haryana) and blood samples of Gojri, Chhattisgarhi, Chilika, Bargur, Belahi, Jhari, and Jersey crossbreed were collected from their respective breeding tract in India.

2.2. DNA isolation and primers used

Genomic DNA was isolated from aseptically collected venous blood using standard phenol/chloroform method with minor modifications (Sambrook and Russell 2001). Quality check and quantification were done by nanodrop spectrophotometer and electrophoresis on 0.8% agarose gel. DNA concentration was determined and samples were diluted 10–40 times (approx. 50–80 ng/µL) with MiliQ water. A 501 bp butyrophilin gene fragment covering part of exon 8 was amplified with a pair of primers, 5'- TGGAGCTCTATGGAAATGGG-3' (forward) and 5'-TACCCAACAGGAAGAAACAG-3' (reverse) with T_M of 60.4°C and 59.5°C, respectively (Taylor et al. 1996a).

2.3. PCR amplification and genotyping conditions

DNA amplification of exon 8 region of *BTN1A1* gene was done by thermocycler. The optimization of PCR was done to get the best possible amplification of product. PCR was carried out in 25 µL reaction volumes consisting of 200 µM of each dNTP, 5 pM of each primer, 1.5 mM $MgCl_2$ and 1.0 U Taq polymerase (Invitrogen, CA). Amplification was standardised in lab using MASTERCYCLER EP (Eppendorf, Germany) with an initial denaturation at 95°C for 4 min followed by 30 cycles of 94°C for 60 s, annealing temperature 58°C for 60 s and 72°C for 60 s, with a final extension at 72°C for 10 min. All samples were screened for *BTN1A1* gene polymorphism using PCR-RFLP test using *HaeIII* restriction enzyme at 37°C for 6 to 8 h and genotyping was done. Genotypes were evaluated by running a small aliquot of PCR-RFLP product on 3% agarose gel.

2.4. Statistical analysis

Gene counting method (Falconer and Mackay 1996) was used for calculating genotype and allele frequencies of exon 8 of *BTN1A1* gene. A chi-square test was also performed to check Hardy-Weinberg equilibrium status in the studied population. All the analysis was carried out using the SPSS (SPSS Inc. 2001).

3. Results and discussion

Genomic DNA isolated from whole blood was of good in quality and concentration. Standardization of the optimum PCR reaction conditions was achieved in the lab and the PCR product of 501 bp was amplified satisfactorily, with repetitions of results at the same amplification conditions among all of the samples of 11 bovine populations studied across India.

Digestion of PCR amplified product by *HaeIII* restriction enzyme revealed three distinct restriction patterns. The first pattern (genotype AA) showed two bands of 316 and 162 bp while the second pattern (genotype BB) showed 283 and 162 bp. The third pattern, a heterozygote (genotype AB) depicted three bands of 316, 283 and 162 bp in agarose gel (Figure 1, 2). Smaller fragments of 23 bp, 56 bp and 33 bp could not be resolved in the gel due to limitations of agarose gel electrophoresis used to revolve the DNA. Thus, this locus revealed the presence of two alleles, namely A and B, in the indigenous and crossbred cattle. Conversely, in the case of the different breeds in the buffalo population allele A was fixed and only band patterns 316 and 162 bp were present and all 340 samples showed the AA genotype (Figure 3).

Among the native Belahi and Jhari cattle, the *BTN1A1* gene was sufficiently polymorphic and showed three expected genotypes, although the BB genotype was found to be less frequent (Table 1). In Tharparkar and Sahiwal cattle, the BB genotype was absent, probably because these populations were sampled from an organized herd where selection is practiced, whereas the Belahi and Jhari cattle could reveal a higher degree of genetic variation as sampling in these cattle was carried out from field herds/breeding tract. Karan Fries cattle, having exotic blood from the Holstein breed, showed all three of the expected genotypes (Table 1), but the BB genotype was rare. In the Jersey crossbreed, the BB genotype was entirely absent. Irrespective of the nature of the sampling of the water buffaloes, all of the five studied populations were monomorphic and had a single genotype (AA).

Allele frequencies for A and B allele were found to be 0.85 and 0.15 in Tharparkar, 0.82, and 0.18 in Sahiwal, 0.84 and 0.16 in Jhari, 0.86 and 0.14 in Belahi, 0.82 and 0.18 in Karan Fries cattle, and 0.83 and 0.17 in Jersey crossbreeds indicating that A is a predominant allele for exon 8 of *BTN1A1* gene among Indian bovines. These were in correspondence with earlier reported frequencies of *BTN1A1* allele as 0.875 and 0.125 (Taylor et al. 1996a), 0.85 and 0.15 (Husaini et al. 1999), 0.84 and 0.14 (Sadr et al. 2008), 0.88 and 0.12 (Komisarek and Dorynek 2003), 0.87 and 0.13 (Bhattacharya et al. 2004), 0.69 and 0.31 (Muszyńska et al. 2010) in different milch bovines. Highest frequency was observed for AA genotype and A allele whereas lowest frequency was found for BB genotype and B allele. Several researchers (Husaini et al. 1999; Zegeye et al. 1999; Badola et al. 2004; Mao et al. 2004) had reported similar results. Milk fat percentage in Indian buffalo breeds averages around 8% whereas it is around 5% in native cattle and in exotic crossbreeds it is around 4% (Vohra and Chakravarty 2011). Thus, this variation

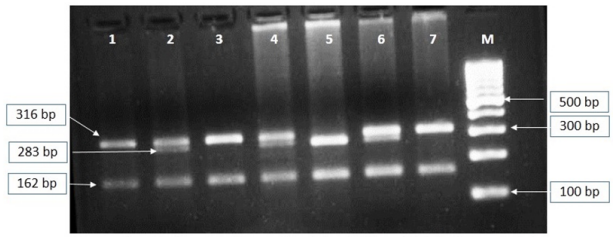

FIGURE 1 Polymorphic *HaeIII* digestion pattern of PCR product (501 bp) in exon 8 of *BTN1A1* gene region in Jersey crossbreed (Lanes 1, 2), Karan Fries (Lane 3, 4), and Jhari (Lane 5, 6, 7) cattle. Lane 1, 3, 7 = AA genotype (product band size 316 bp and 162 bp); Lane 2, 4, 6 = AB genotype (product band size 316 bp, 283 bp, and 162 bp); Lane 5 = BB genotype (product band size 283 bp and 162 bp); Lane M = 100 bp ladder.

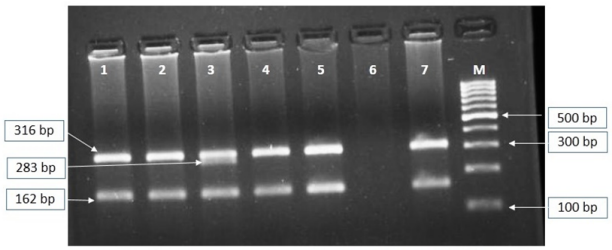

FIGURE 2 Polymorphic *HaeIII* digestion pattern of PCR product (501 bp) in exon 8 of *BTN1A1* gene region in Sahiwal (Lanes 1, 3), and Tharparkar (Lane 4, 5, 6) cattle. Lane 1, 2, 4, 5, 6 = AA genotype (product band size 316 bp and 162 bp); Lane 3 = AB genotype (product band size 316 bp, 283 bp, and 162 bp); Lane M = 100 bp ladder.

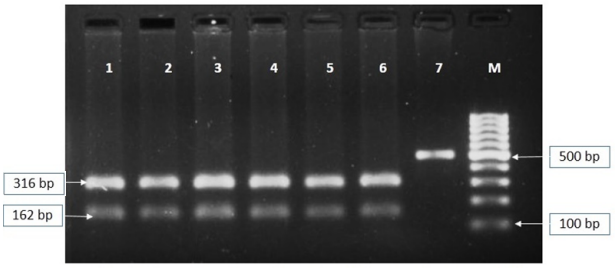

FIGURE 3 Monomorphic *HaeIII* digestion pattern of PCR product (501 bp) in exon 8 of *BTN1A1* gene region in Murrah (Lanes 1, 2), Gojri (Lane 3, 4), and Chhattisgarhi (Lane 5, 6) buffaloes. Lane 1 to 6 = AA genotype (product band size 316 bp and 162 bp); Lane 7 = uncut PCR product as control (501 bp); Lane M = 100 bp ladder.

TABLE 1 Genotype and allele frequencies of BTN1A1 gene in Indian milch bovines.

Name of population	Distribution (state)	Sample size	Genotype frequency			Allele frequency	
			AA	AB	BB	A	B
Indigenous cattle							
Tharparkar	Rajasthan	30	0.70	0.30	0.00	0.85	0.15
Sahiwal	Rajasthan, Punjab	30	0.63	0.37	0.00	0.82	0.18
Jhari	Telangana	30	0.70	0.27	0.03	0.84	0.16
Belahi	Haryana, Chandigarh	48	0.76	0.20	0.04	0.86	0.14
Total		138	0.69	0.29	0.02	0.84	0.16
Crossbred cattle							
Karan Fries	Haryana, Punjab	30	0.67	0.30	0.03	0.82	0.18
Jersey crossbreed	Himachal Pradesh, coastal parts	30	0.65	0.35	0.00	0.83	0.17
Total		60	0.66	0.33	0.01	0.83	0.17
Water buffalo							
Murrah	Haryana	200	1.00	0.00	0.00	1.00	0.00
Gojri	Punjab, Himachal Pradesh	40	1.00	0.00	0.00	1.00	0.00
Chhattisgarhi	North & Central Chhattisgarh	40	1.00	0.00	0.00	1.00	0.00
Chilika	Odisha	30	1.00	0.00	0.00	1.00	0.00
Bargur	Tamil Nadu	30	1.00	0.00	0.00	1.00	0.00
Total		340	1.00	0.00	0.00	1.00	0.00

in *BTN1A1* gene i.e. **A** allele could be one of the allelic marker associated with high milk fat in Indian bovines.

Chi square (χ^2) test was used to evaluate Hardy-Weinberg equilibrium (HWE) in the studied populations. Populations followed Hardy-Weinberg equilibrium in indigenous and crossbred cattle with χ^2 values 0.879, 1.39, 0.103, 0.124, 0.819, and 0.007 in Tharparkar, Sahiwal, Jhari, Belahi, Jersey crossbreed, and Karan Fries cattle, respectively. Overall χ^2 value of different breeds of indigenous and crossbreed cattle was 2.321. The calculated chi-square values were found to be less than tabulated values at 1% (9.21) and 5% (5.99), indicating non-significant values implying that HWE was maintained in the populations. Thus it can be inferred that so far the studied population of indigenous and crossbred cattle followed random mating and has not been subjected to selection with respect to *BTN1A1* gene in Indian bovines.

4. Conclusions

BTN1A1 is possibly a candidate gene affecting fat percent, an economically important trait, in dairy bovines. It may be inferred from our findings that *BTN1A1* gene is variable in cattle and can be successfully be detected through PCR-RFLP based test. Indian water buffalo seems to have been fixed for **A** allele as the locus is monomorphic across the breeds of buffalo in India. Further it is hypothesised that early selection based on **A** allele of exon 8 of *BTN1A1* gene could serve as one of the essential SNP for genomic chip/genetic marker based selection strategy if adopted for Indian cattle.

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Authors' contributions

VV designed the study. MK, PR, RD carried out the laboratory work. AC analyzed the data. MK and VV wrote the manuscript. All authors read and approved the final version of manuscript.

Competing interests

There are no competing interests.

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