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Expression of Coat Colour Gene, *MC1R* in Cattle

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ABSTRACT

Background: Scanty reports are available in the literature about the coat colour inheritance and its related genes in cattle. Therefore, an experiment was conducted to know the expression of coat colour gene, *MC1R* in cattle.

Methods: DNA was extracted from 85 whole blood samples of Red Chittagong cattle (RCC), Non-descriptive deshi and their crossbred of which 75 positive samples were sequenced by Sanger sequencer. Sequence alignment, pair and multi-alignment comparison of the *MC1R* gene of different genotype and a phylogenic tree constructed by MEGA6 software.

Result: RCC has a dominant R gene for red colour. Point mutation was observed at 954 bp and substitution (395G→A) was found in the *MC1R* gene of RCC genotype. The evolutionary history of branching pattern showed the relatedness of *MC1R* nucleotide sequences of cattle and this gene have been regulating coat colour inheritance of cattle.

Key words: Gene, Genotype, Mutation, Sequencing.

INTRODUCTION

Cattle genetic resources of Bangladesh mostly consist of native cattle. This includes Red Chittagong cattle (RCC), Pabna cattle, North Bengal Grey cattle, Munshiganj cattle, Non-descriptive deshi, crossbred cattle and a few additional exotic pure breeds namely Holstein- Friesian, Sahiwal, Sindhi and Jersey (Khan, 2009; Bhuiyan, 2013). Importantly, RCC, Pabna cattle, North Bengal Grey cattle and Munshiganj cattle are the four prominent local varieties.

Moreover, amongst the indigenous variety of cattle, RCC is distinct due to its unique characteristics such as red coat colour, body features and productivity. Coat colour is one of the most important phenotypic and economic traits in animal and is mainly used for breed identification and characterization (Gebreselassie *et al*., 2020).

Several genes such as *MC1R, KITLG, ASIP* and *LYST* genes are responsible for the coat colour in animals (Hartatik, 2017). The *MC1R* gene is reported as a potential candidate gene that plays a significant role in melanogenesis and pigmentation and is responsible for black coat colour in mammals (Switonski *et al*., 2013) and the *PMEL* variant is associated with white color, known as silver-dun (Schmutz *et al*., 2013) and *PMEL* mutation for diluting the black coat color in cattle (Laible *et al*., 2021). In addition, coat colour does not only play for the appearance of the animal, it also impacted the survival, production and reproduction. For example, the white coat cows (Holstein) produce more milk than black coat cows (Holstein) in the first generation (Becerril *et al*., 1993) and light colour animals gained more weight than dark ones (Finch *et al*., 1984). Furthermore, Gebreselassie *et al*., (2020) stated that the *MC1R* gene expression level was significantly higher in black-head Tan sheep than in white sheep. Such studies, however, have failed to address the association between coat colour inheritance. A comprehensive study on coat colour inheritance and its relationship with production traits is essential for improving the breeding efficiency and undertaking the structured genetic improvement programme. Therefore, the current study aims to contribute to this growing area of research by identifying the effects of the *MC1R* gene on the coat colour inheritance of cattle variety of Bangladesh.

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MATERIALS AND METHODS

Ethics and experimental period

The study was conducted under the Department of Genetics and Animal Breeding of Chattogram Veterinary and Animal Sciences University (CVASU), from January 2018 to March 2019 following the animal ethics rules and decisions of the ethics committee of CVASU (Memo no.-CVASU/Dir (R&E) EC/2020/165(8), Date 09/03/2020). The field experiment was completed in the Hathazari Upazila (sub-district) of the Chattogram district and molecular study at the Poultry Research and Training Center (PRTC) of CVASU, Bangladesh.

Experimental animals

The genetic group of animals selected for the experiment was Red Chittagong cattle (RCC, red coat colour), non-

descriptive (ND, brown coat colour) and crossbred (RCC×ND, reddish-brown).

Blood collection and primer designing for molecular study

Eighty-five blood samples were collected from the Jugular vein of the three genotypes of cattle [RCC (N=30), ND (N=35] and crossbred [RCC × ND, N=20)] in a microtube containing 0.5 M EDTA (as anticoagulants) and was stored in -20°C. Gene (*MC1R*) was designed as a forward primer - 5′- GGA CCC TGA GAG CAA GCA C-3′; reverse primer - 5′- CTC ACC TTC AGG GAT GGT CTA-3′; amplified region: exon 1; chromosome no. 18, consists of a single exon of 954 (bp) according to Hartatik (2017).

DNA extraction and Polymerase chain reaction (PCR)

The FavorPrep™ blood genomic DNA extraction mini kit was used for extracting DNA from the whole blood samples. A total of 30 µL reaction mixture (1 µL DNA (10-100 ng), 1.5 µL of each primer (10 pmol μL^{-1}), 15 µL PCR kit (KappaFast2G, Biosystem, USA) and 12.5 µL aquabidest) was used for PCR reaction. The 296 bp *MC1R* gene fragment was amplified using 30 amplification cycles (Peqlab, Germany) as the conditions of initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 94°C for the 30s, annealing at 57°C for 30s and extension at 72°C for 30s was followed by a final extension at 72°C for 10 min.

Each amplification product was analyzed by electrophoresis on a 1% agarose gel in a TBE buffer. The DNA bands were stained with ethidium bromide to visualize by UV light. Then the PCR product was purified.

Gene sequencing

The purified PCR products (75 positive samples; RCC (N=25), ND (N=32) and crossbred [RCC × ND, N=18)] were Sanger-sequenced (for both forward and reverse strands) at the Macrogen sequencing facility (Macrogen Inc., Seoul, Korea) using ABI PRISM 3730XL Analyzer (96 capillary types). Using the BLAST tool (available at http://ncbi.nlm.nih.gov) explored similar sequences to find out a reference sequence with the aim to investigate possible mutations.

Statistical analysis

The DNA sequences of PCR product (were aligned by using MEGA 6 (Clustal W) with the reference sequences (KP182069.1) in order to find mutation. Though both forward and reverse strand were sequenced, here only reverse sequence was used as it showed better chromatogram (reverse complement of the reverse sequence to make it forward using MEGA6 software). The chromatogram of gene sequence indicates three different genotypes (RR, Rr and rr) for three different phenotypes. The allele (expected) frequency was calculated following the Hardy-Weinberg Equilibrium.

The nucleotide sequences were analyzed to identify single nucleotide polymorphism, evolutionary relationships and maximum composite likelihood estimation using software such as NCBI BLAST (Johnson *et al*., 2008; Madden, 2013) and MEGA6 software (Tamura *et al*., 2012). Statistical significacnes were analysed using the chi-square test by examining the goodness of fit tests between frequencies of the observed and expected occurences.

RESULTS AND DISCUSSION

A total of 85 samples were tested for the presence of the *MC1R* gene. Of these 75 (88.2%) were tested positive, while the rest of the samples were negative, probably due to damaged DNA.

Genotypes and frequency of MCIR gene

Allelic frequencies of the *MC1R* gene with the different coat colours of cattle and chi-square value are shown in Table 1. The RCC cattle were a more dominant genotype (RR) for red colour than ND and crossbred (Table 1), this finding agreed with the results of Indonesian cattle (Hartatik, 2017). The low frequency of the *MC1R*, r allele might be due to the low actual r allele frequency in the population. Similar results of the *MC1R* gene study were reported for Chinese yakow cattle (XiD *et al*., 2012) and Datong yak (Peng *et al*., 2020). The pigmentation in the colour of cattle may be affected by the *MC1R* gene, which forms melanocytes, it stimulates tyrosinase to produce eumelanin which is responsible for brown to black colour. The estimated Chi-square value was compared with the tabulated value [5.99; $p<0.05$], this value confirms the distance between alleles and the expected with the observed values were matched with the Hardy-Weinberg equilibrium.

Sequence analysis and mutations of MC1R gene in RCC, ND and crossbred cattle

The sequences of the *MC1R* gene in the cattle showed a lot of similarity among the species being studied in the locus of

Table 1: Observed and expected genotype frequencies, allelic frequencies of the *MC1R* gene with coat colour of cattle and Chi-square (χ^2) test value.

Genotypes	N	Genotype observed			Genotype (%)			[1]Allele frequencies		Genotype expected			χ^{2} [2]
		RR	Rr	rr	RR	Rr	rr	R	r	RR	Rr	rr	
RCC	25	21	4	0	84	16	0	0.92	0.08	21.16	3.68	0.16	0.91
ND	32	22	7	3	68.7	21.9	9.4	0.80	0.20	20.48	10.24	1.28	0.18
Cross-bred	18	10	5	3	55.6	27.7	16.7	0.69	0.31	8.57	7.70	1.73	0.35

[1]R is for red and r is for brownish colour, RCC (red) = Red Chittagong cattle, ND (brownish) = Non-descriptive deshi; Crossbred (reddish-brown) = RCC × ND.

[2]χ^2= chi square, Tabulated value of χ^2 is 5.99 at 5% level of significant in 2 degrees of freedom.

sequence alignment analysis occurred at position 406 compared with reference Gene Bank accession number KP182069.1 (Fig 1.1). Based on the sequence resulting from *MC1R* 390-400bp, the sequence 390-AAGAAGCCA-400 for RCC (Fig 1.2a), the sequence 390-CGTAGAAAA-400 for ND cattle (Fig 1.2b) and the sequence 390-AAGAGGCCA-400 for crossbreed (Fig 1.2c) can be compared wherein chromatograms are separately chracterized. Moreover, the sequence result of *MC1R* gene at position 395 bp (Fig 1.2a,c) indicated two genotypes: Heterozygote (A/G) for RCC and homozygote (G/G) for crossbreds. Thus, the mutation A instead of G was as substitution 395G→A. Sequence alignment analysis indicates changes of sequence order occurred at position as substitution 398G→A in ND cattle (Fig 1.2b) exhibiting heterozygote (G/A) whereas RCC (Fig 1.2a) is normal homozygous (C/C) of dominant sequence.

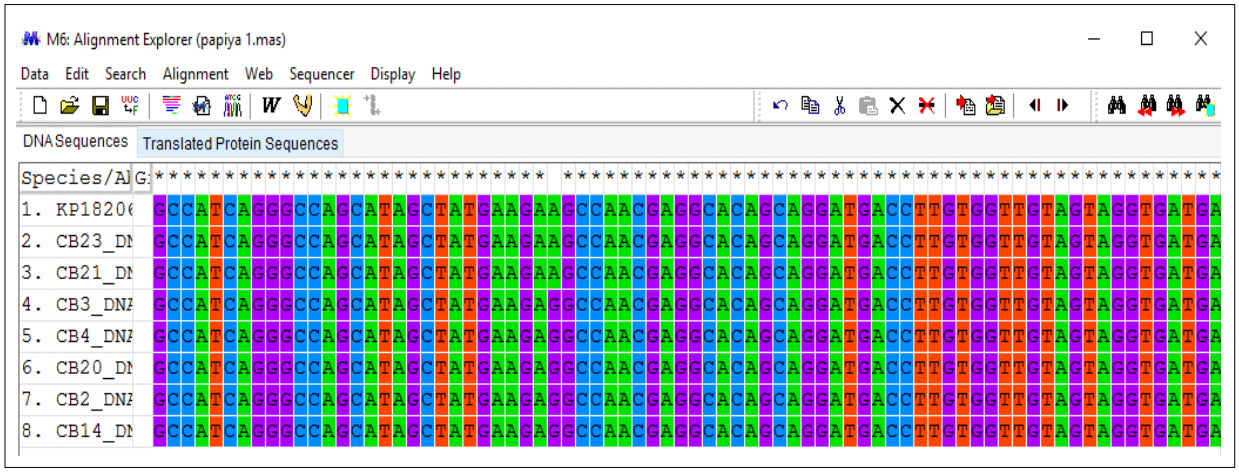

Fig 1.1: Sequence alignment with reference sequence at position 406 compared with GeneBank accession number KP182069.1 for MC1R gene from BLUST MEGA 6 program.

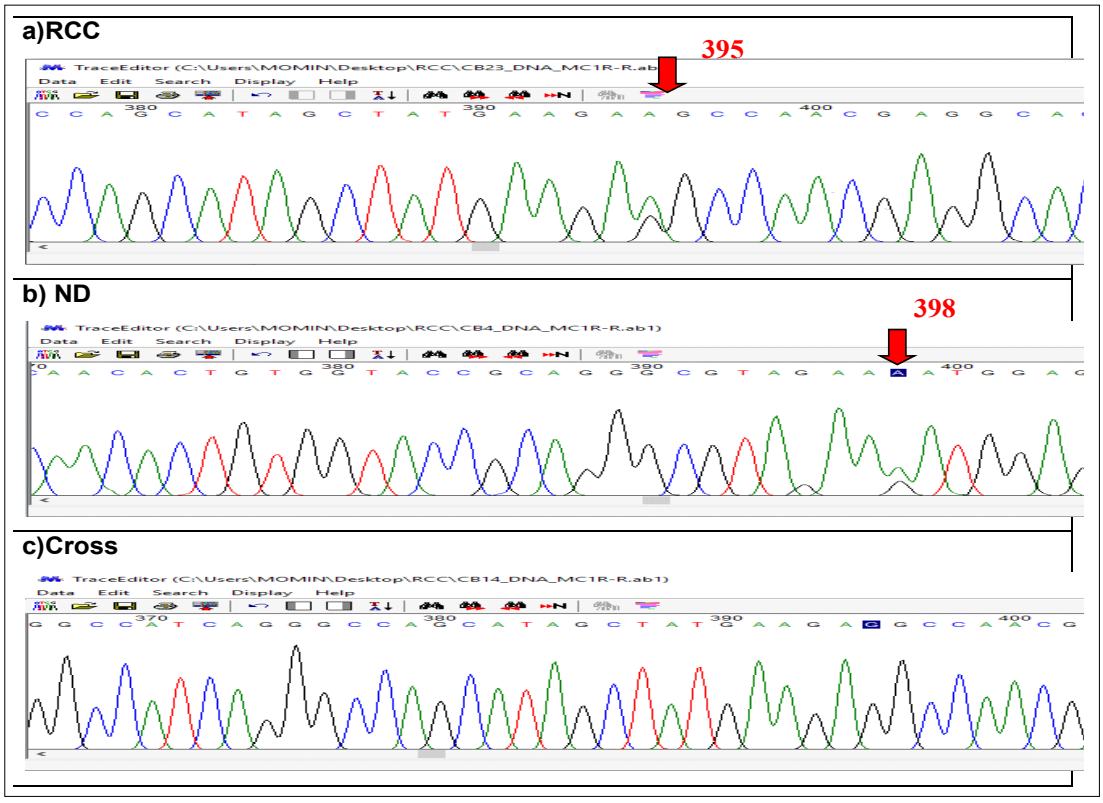

Fig 1.2: Chromatogram of the sequence, the sequence 390-AAGAAGCCA-400 for RCC (Fig 1.2a), the sequence 390-CGTAGAAAA-400 for Non-Descriptive (ND) cattle (Fig 1.2b) and the sequence 390-AAGAGGCCA-400 for crossbreed (Fig 1.2c).

The sequence analysis showed that the dominant red (RCC) and the reddish (ND) allele were exclusively associated with an insertion and deletion of amino acids in the *MC1R* transmembrane region. The *MC1R* gene mutations in any mammal's produce dark or black coat colour (Fontanesi *et al*., 2009) and due to mutations of those genes, produces red-yellow coat colour. The results shows that the mutation of *MC1R* gene sequence in ND was reddish (red-yellow) and RCC was red coat colour. Similarly, depending on the degree of interbreeding between the different colour phenotypes (which is assumed to be highly likely), the colour loci could be linkage disequilibrium with the production-related genes.

Nucleotide sequences comparison of *MC1R* gene

The scoring of similar and matching rate of different sequences of different coat colour cattle is shown in Table 2. The maximum similarities were found in the sequence of *Bos indicus* Clone23 (NCBI accession no: MG373747.1) for all genotypes which was 98% similar (Table 2). The maximum score of genotypes was similar, except RCC with the crossbred (*Bos indicus* × *Bos taurus*) *MC1R* (mRNA) (NCBI accession no: XM02751377.2) which was 1570. Besides, the E value and identity for all genotypes was similar with the sequences of *Bos indicus* × *Bos taurus MC1R* (mRNA) (NCBI accession no: XM02751377.2), *Bos indicus* (*MC1R*) NICRA-12 (NCBI accession no: P182060.1), *Bos indicus* (*MC1R*) clone23 (NCBI accession no: MG373747.1) and Cattle MSH receptor mRNA-1182 (NCBI accession no: S71017.1). Despite many similarities, the sequences at some sites had a base variation and replacement too.

Evolutionary relationship taxa of MC1R gene

The results of the evolutionary analysis are shown phylogenetic tree is drawn to scale in Fig 2, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. On the eve of *MC1R* gene nucleotide sequence, *Bos indicus* (NCBI accession no: KP182069.1) was closely related to CB14 DNA *MC1R-R* than the sequence CB4 DNA *MC1R-R* and CB23 DNA *MC1R-R*. On the other hand, the *MC1R* gene nucleotide sequence of CB4 DNA *MC1R-R* (ND, Reddish) and CB23 DNA *MC1R-R* were closely related to the nucleotide sequence of *Bos taurus* breed, Karan Fries (NCBI accession no: MG373684) compared to others.

The taxa relatedness were interpreted by most recent common ancestry. Hubbard *et al*., (2010) stated that taxa that share a more recent common ancestor were more closely related to each other than the taxon with a less recent common ancestor. A possible explanantion for this might be that several phylogeny results indicated that the *MC1R* gene is greatly conserved among vertebrates and variations are closely associated with pigmentation differences (Gebreselassie *et al*., 2020; Nunes *et al*., 2011; Nei and Kumar, 2000). The designated *MC1R* (CB14)

Table 2: The scoring of similarity and matching rate of different sequences of different coat colour cattle genotype.

	Genotype								
	Red (RCC)			Reddish (ND)			Brownish (Crossbred)		
	Max. score	E value	Identity	Max. score	E value	Identity	Max. score	E value	Identity
Bos indicus × *Bos taurus MC1R* (mRNA) × M02751377.2	1570	0.0	96.29	1452	0.0	96.29	1452	0.0	96.29
Bos indicus (*MC1R*) NICRA-12P182060.1	1419	0.0	97.05	1419	0.0	97.05	1419	0.0	97.05
Bos indicus (*MC1R*) Clone 23MG373747.1	1570	0.0	98.65	1570	0.0	98.65	1570	0.0	98.65
Cattle MSH receptorm RNA-1182S71017.1	1425	0.0	95.96	1425	0.0	95.96	1425	0.0	95.96

(1) Genotype description presented in Table 1.

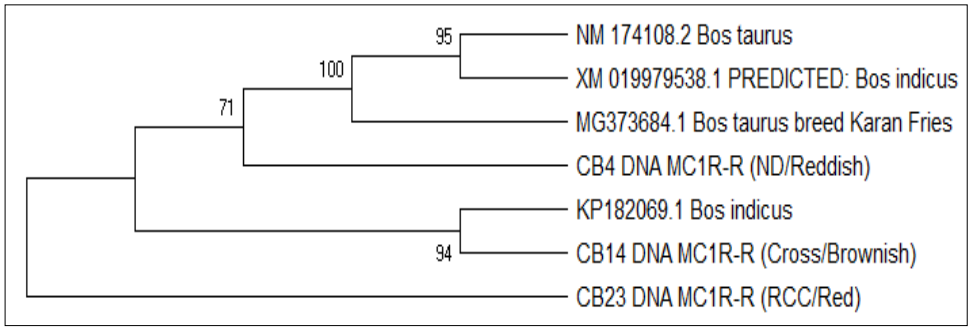

Fig 2: Phylogenetic tree drawn based on nucleotide sequences of the MC1R gene.

sequences were closely related to the sequence of *Bos indicus MC1R* (NCBI accession no: KP182069.1). This close relation in gene sequences may be due to the same environmental conditions and the common ancestor. There are, however, other possible explanantions for the genetic diversity that relates to adaptation of changing environmental conditions and have positive implications to a population (Das and Choudhury, 2021).

CONCLUSION

The relevance of the *MC1R* gene in regulating the coat colour inheritance of cattle is clearly supported by the current findings. In general, therefore, it seems that the genomic thought will be helpful to conserve the genetic constituents of cattle. The findings reported here shed new light on the application of the genetic improvement of cattle. However, to know the potential role of all the possible mutations in the *MC1R* gene on coat colour inheritance of cattle, more investigations are needed to establish a greater degree of accuracy and perceive the complexities of the genome.

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Conflicts of interest

There was no conflicts of interest with the funding body and others.

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