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Genetic Characterization Of Red Chittagong Cattle Population Using Y Chromosomal Haplotypes And Polymorphism Of Genes Associated With Economic Traits And Coat Color

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Abstract

Red Chittagong (RC) is a breed of cattle that is native to Bangladesh, and is known for its adaptation to tropical climates. The Y chromosomal haplotypes formed by 5 single nucleotide polymorphisms (SNPs) of sex-determining region Y gene (SRY) in Y chromosome shows the mode of paternal inheritance in cattle. The polymorphism of stearoyl- CoA desaturase (SCD) and sterol regulatory element- binding protein- 1 (SREBP-1) genes are associated with fatty acid composition in meat and melanocortin receptor-1 (MC1R) gene is involved in coat color determination in cattle. The aim of this study was to investigate genetic characteristics of these cattle using Y chromosomal haplotypes and polymorphism of genes associated with economical traits and coat color. Therefore, we investigated Y chromosomal haplotypes and genotyped SCD, SREBP-1 and MC1R genes by sequencing in these cattle population. The sequence analysis of the Y chromosome haplotype indicates, in addition to zebu haplotype, taurine haplotype also exists in RC cattle, suggesting gene flow from taurine cattle, that will important for genetic characterization of these cattle. The genotyping results of the SCD, SREBP-1, and MC1R genes show that these genes are polymorphic in the population. Particularly, the presence of the desirable alleles of the SCD and SREBP-1 gene will be important for selection of these cattle for meat productivity. This finding will be helpful in future conservation and breeding of the RC cattle population.

Key Word: *SRY, MC1R, SCD, SREBP-1, and Genotyping*

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I. INTRODUCTION

Most of the indigenous cattle in Bangladesh are of *Bos indicus* type including Red Chittagong (RC) cattle. The observed coat color of the RC cattle is red and has some other distinguishable characters which make them unique to the other cattle breeds. Their productive and reproductive performances are relatively better as compared to other available indigenous cattle of Bangladesh[2, 4]. Attractive coat color with delicious milk and meat compared to other indigenous cattle made peoples first preference to have RC cattle in their family[5].

Mitochondrial DNA (mtDNA) haplotypes that clarify the maternal origin of various cattle populations have indicated that the RC cattle belongs to zebu cattle and introgression of taurine cattle has occurred in these cattle[1]. The Y chromosomal haplotype formed by 5 single nucleotide polymorphisms (*SNPs*) of sex-determining region Y gene (*SRY*) in Y chromosome in cattle that shows mode of paternal inheritance have been used for phylogenetic analysis of populations of livestock paternal lines for classification into different types e.g. taurine, zebu, and banteng types[11]. But little is known about Y chromosomal haplotypes in RC cattle. Therefore, here we have investigated Y chromosomal haplotypes in these cattle by direct sequencing. The investigation of the distribution of the Y chromosomal haplotypes will be informative for genetic characterization of these cattle.

The polymorphisms of genes associated with economical traits such as fatty acid composition in meat, and coat color variations, have been identified in cattle of different breeds, but there is also scarcity of information about these polymorphisms in RC cattle. Therefore, we have investigated whether these polymorphisms are existing in RC cattle and in this study, we have genotyped the polymorphisms of stearoyl- CoA desaturase (*SCD*), sterol regulatory element- binding protein- 1 (*SREBP-1*) and Melanocortin Receptor-1 (*MC1R)* genes in these cattle by direct sequencing of the PCR products. *SCD* and SREBP-1 genes are associated with fatty acid composition in meat, and A allele of *SCD* and S allele of *SREBP-1* increase monounsaturated fatty acids of fat in meat that is beneficial for health[3, 17.] *MC1R* gene is involved in coat color

determination and e, E+, and E^D alleles are associated with brown, dark brown and black coat color, respectively[9]. Since, these polymorphisms have been identified in the taurine cattle, distribution of these polymorphisms in the zebu cattle including RC cattle will be important for selection of RC cattle for meat productivity.

II. MATERIALS AND METHODS

Total 15 blood samples (7 Males and 8 Females) were randomly collected from the population of RC cattle from Dairy Farm, Bangladesh Agricultural University, Mymensingh, Bangladesh. The extraction of DNA from whole blood was performed according to the method[20], using DNA Extraction kit (Promega Corporation, WI, USA). For genotyping by sequencing, *SCD*, *SREBP-1*, *MC1R* (E^+, E^D), and *MC1R* (E, e) gene were amplified by PCR using the primer pair listed in Table 1[10, 15]. PCR reactions were performed in 10 µl reaction mixtures containing 20 ng of genomic DNA, 0.2 µM primers, 0.2 µmol/L dNTP, 5 × PCR buffer, and 0.5 U Go Taq DNA polymerase (Promega Corporation, WI, USA), for 35 to 40 cycles of denaturation at 94°C for 30-120 sec, annealing at the temperatures indicated in Table 1 for 30-60 sec, and extension at 72°C for 30-120 sec. After PCR amplification, PCR products were electrophoresed in an agarose gel in TAE buffer, stained with ethidium bromide, and visualized using UV transilluminator. Furthermore, the amplified fragments were directly sequenced by using these primers. To determine the Y chromosomal haplotypes, a 1062-bp segment of *SRY* gene in the male-specific region of the cattle Y chromosome were amplified by using pairs of primer listed in Table 1[11] and the amplified fragments were directly sequenced by using these primers. PCR reaction were carried out in 10 µl reaction mixtures containing 10 ng of genomic DNA, 0.2 µM primers, 0.2 µmol/L dNTP, 5 × PCR buffer, and 0.5 U KOD FX Taq polymerase (Toyobo, Osaka, Japan), for 35 cycles of denaturation at 94°C for 15 sec, annealing at 58°C for 30 sec, and extension at 72°C for 45 sec[18].

Table 1: Primer sequences, fragment lengths and annealing temperatures

Gene	Primer sequences (5' to 3')	Length (bp)	Annealing Temperature (^{0}C)
SCD	F-GTGTCCTGTTGTTGTGCTTCATCCTGCC R-AATATTCTCTCGGGGGTTGATGGTCTTG	197	60
SREBP-1	F-CCACAACGCCATCGAGAAACGCTAC R-GGCCTTCCCTGACCACCCAACTTAG	348	65
MC1R (E^+, E^D)	F-AACCTGCACTCCCCCATGTACTACT R-ACATTGTCCAACTGCTGCACCACGG	154 96, 58	65
MC1R (E, e)	F-ATCTGCTGCCTGGCTGTGTCTGACT R-GGCGTAGAAGATGGAGATGTAGCGG	219	65
SRY	F-CCGGGCTATAAATATCGACCTC R-GATGAAACCTTGGGTCTCACAG	1,062	58

III. RESULTS AND DISCUSSIONS

We determined the Y chromosomal haplotypes of the RC cattle by sequencing the 1062 bp segment of the SRY gene on Y chromosome. Since, the Y chromosomal haplotype of cattle can be classified into taurine, zebu, and banteng types by 5 *SNPs* in the mentioned segment[11], but the present result indicates that, 6 out of 7 male cattle possesses zebu type Y chromosomal haplotype, and the remaining one has taurine type haplotype, and no banteng type haplotype was observed (Table 2). Previous report showed that some zebu type cattle breeds of South and Southeast Asian countries including RC cattle have trace of introgression of taurine genome in autosomes, mtDNA, and Y chromosome[1, 8]. Therefore, our findings of the presence of the taurine type Y chromosomal haplotype in RC cattle is the first report showing the paternal introgression of taurine genome into the RC cattle populations and will be informative for considering the origin and history of RC cattle.

Next, we investigated the polymorphisms of the genes associated with economic traits and coat color of cattle including *SCD*, *SREBP-1* and *MC1R* genes in the RC cattle by direct sequencing. As the results of the genotyping, three genotypes AA, AV, VV of *SCD* and SS, LS, LL of *SREBP-1*, and two genotypes E^+E^+ and E^+e of *MC1R* were observed, indicating that both two alleles of these genes are present in the RC Cattle population.

Table 2: Haplotypes of SRY gene on Y chromosome of 7 RC cattle

Haplotype	Position

	1	2	2	2	2
	7	0	1	1	3
	4	5	0	4	7
	8	9	0	4	2
DQ336527[a]	T	A	T	T	C
DQ336526[b]	G	.	C	.	T
DQ336528[c]	.	G	.	C	N[d]
RC cattle (N = 6)	.	.	.	.	.
RC cattle (N = 1)	G		C		T
Reference sequences for [a]*Bos indicus*, [b]*BosTaurus* and [c]*Bos javanicus*. [d]This position is not included in DQ336528.					

The frequencies of the A allele of *SCD* gene, S allele of *SREBP1* gene and e allele of *MC1R* gene were 0.13, 0.17 and 0.06, respectively (Table 3), suggesting that the desirable allele of *SCD* and *SREBP-1* and mutant type allele of *MC1R*, are present in the population of RC cattle at lower frequency. The observed distributions of the genotypes are not significantly different from those expected from Hardy-Weinberg Equilibrium. Since, the polymorphisms of the *SCD* and *SREBP-1* genes have been reported to be associated with fatty acid composition of meat and the animals possessing the A allele of *SCD* and S allele of *SREBP-1* showed increased mono-unsaturated fatty acid composition of meat fat. The present findings suggest the possibility that the polymorphisms of these genes, which were originally identified in taurine type cattle, were also present in zebu type cattle. Although the presence of these alleles in RC cattle can be caused by introgression of taurine genome as observed in the Y chromosomal haplotype, similar findings of the presence of the taurine alleles in zebu cattle has also been reported[7, 10, 12, 13, 15, 21]. Therefore, presence of the desired alleles of these genes at relatively lower frequency in the population of RC cattle might be effective and hence may be considered for improving the meat quality of the population by future selection programs.

Table 3: Genotype distributions and allele frequencies of the three genes associated with economical traits and coat color.

Gene	N	Genotype frequencies						Allele frequencies		Chi square values for HWE test
SCD	15	AA		AV		VV		A	V	2.68
		0.07		0.13		0.80		0.13	0.87	
SREBP-1	15	LL		LS		SS		L	S	1.17
		0.73		0.20		0.06		0.83	0.17	
MC1R	15	E^D/E^D	E^D/E^+	E^+/E^+	E^D/e	E^+/e	e/e	E^D	E^+ *e*	
		0	0	0.87	0	0.13	0	*0*	0.94 0.06	0.07

*Significantly deviation from Hardy Weinberg Equilibrium (HWE) (P < 0.05)

Generally, the coat color of RC cattle is Red. The higher frequencies of E^+E^+ genotype was for red coat color (Table 3), this finding agreed with the results of Indonesian cattle and RC cattle[6, 16]. The lower frequency of the E^+e genotype (Table 3) might be due to the lower presence of actual E^+e genotype in these populations. Similar results of the *MC1R* gene study were reported for Laotian cattle[13], Datong yak[14], Indonesian Pisisir cattle[15] and Chinese yakow cattle[19]. The pigmentation in the color 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 color. Our findings of lower frequency of E^+e genotypes of *MC1R* in RC cattle indicate that the common Red coat color is not caused only by the mutation of *MC1R* gene identified in taurine cattle. In the taurine cattle, animals possessing E^+ allele of *MC1R* (E^+ E^+ or E^+ e) show dark brown to black coat color and animals homozygous for e allele (ee) show light brown to red coat color. Further investigation of coat color related genes including *TYRP1*,*TYRP2*, and *ASIP* genes in addition to *MC1R* gene is necessary to uncover the genetic base of coat color determination in zebu cattle including RC cattle.

IV. CONCLUSION

This is the first report for a genetic characterization of RC cattle using Y chromosomal haplotypes and polymorphism of genes associated with economical traits and coat color. The findings of the presence of Y chromosomal haplotypes and distributions of genes and genotypes associated with economical traits, and coat color will be informative for understanding the genetic characteristics of the Red Chittagong Cattle.

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