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Study on candidate genes for milk production traits of Red Chittagong Cattle

M.P. Mostari* and M.Y.A. Khan

Animal Production Research Division
Bangladesh Livestock Research Institute, Savar, Dhaka.

Abstract

The study was carried out on *Stearoyl-CoA desaturase (SCD,) diacylglycerolacyltransferase-1 (DGAT1) and ATP-binding cassette G2 (ABCG2)* genes which are responsible for variation in milk production traits (milk yield, fat yield, protein yield, and SNF yield) in cattle. These genes were used as candidate genes in Red Chittagong Cattle (RCC) breed of Bangladesh Livestock Research Institute (BLRI) herd for detection of single nucleotide polymorphisms (SNPs) causing variation in milk production traits. Focusing on the effects of SNPs on milk production traits, phenotypic variation within RCC breed was identified and categorized based on milk production traits. Average lactation yield varied from 527 to 1436 kg (n=29) per lactation. About 18% of lactating cows showed an average of >1000 kg per lactation. Average fat percent ranged from 4.71 to 6.25 (n=15). Eighteen (18) set of primers were designed to amplify targeted regions of *SCD, DGAT1* and *ABCG2* genes, where 8 set from DGAT1, 6 set from SCD and 4 set from *ABCG2* gene. Pooled DNA from 50 RCC cows and 5 RCC bulls were used in sequencing. In sequence analysis, the *SCD, DGAT1* and *ABCG2* alleles found fixed in RCC. This study suggests an evidence that RCC breed has fixed alleles with respect to *SCD, DGAT1* and *ABCG2* genes reported to be responsible for higher milk fat yield, higher fat and protein percent.

Keywords: Red Chittagong Cattle, milk production, *SCD, DGAT1,* and *ABCG2* genes.

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Introduction

The primary goal of researchers in the development of dairy industry is to increase milk production without reducing the quality of milk. But dairy cattle have a lengthy generation interval, it is time-consuming and expensive to perform progeny tests, and many have low heritability. In a progeny-testing programme, the accuracy of selection depends largely on the number of off spring per sire and, hence, on the number of cows in progeny test herds available for mating to young unproven bulls. In classical and conventional genetics, selection is based on phenotypes, without knowing which genes are being selected. The development of molecular markers was, therefore, a major breakthrough.

To achieve sustainable genetic improvement, it is necessary to have a population with

*Corresponding author: adiba_blri@yahoo.com

genetic variation and a tool for selection. The most efficient method of genetic selection is with the use of gene-assisted selection, which involves the identification of functional single nucleotide polymorphisms (SNPs) responsible for changes in phenotypes.

Marker-assisted selection (MAS) is an indirect method of selection, where a trait of interest is selected based on a marker (morphological, biochemical or DNA/RNA variation) linked to a trait of interest (productivity, disease resistance, abiotic stress tolerance, and quality), rather than on the trait itself. Identification of genes that are in quantitative trait loci (QTL) can provide the accurate markers for MAS in livestock population. Therefore, MAS is thought to be very useful in the dairy industry (Ruane *et al*., 1996, Boichard *et al.*, 2006). However, potential SNPs in some candidate genes have been reported for their association with milk production traits. Candidate genes underlying this variation may be found in milk production and metabolism pathways, which are under the control of multiple genes. Single Nucleotide Polymorphisms in the *diacylglycerolacyltransferase1 (DGAT1), stearoyl-CoA desaturase 1(SCD1), ATP-binding cassette G2 (ABCG2), fatty acid synthase (FASN), oxidized low-density lipoprotein receptor 1 (OLR1), prolactin (PRL), signal transducer activator of transcription 5A (STAT5A) and growthhormone receptor (GHR)* genes have been shown to affect the composition of bovine milk in different cattle populations (Schenninket *et al.*, 2009, Sunet *et al.*, 2009, Alim *et al.*, 2012). This study was undertaken to detect SNP sin DGAT1, SCD and ABCG2 genes against to observed phenotypic variation in milk production traits of RCC, and to use of potential SNPs responsible for higher milk production in MAS.

Materials and Methods

1. Phenotypic data recording on milk production traits of RCC

RCC herd consists of about 50 cows were at different lactation stages and dry cows. Phenotypic data on milk yield collected from daily milk yield record book and milk traits (fat yield, protein yield, SNF yield, and lactose yield) were recorded from milk sample analysis using LACTOSTAR (Milk component analyzer, Model 3510) in every 15 days interval. The milk yield and milk sample analysis data were used in Microsoft Office Excel 2007 program to analyze and identify phenotypic variation in RCC for association study with DGAT1, SCD, and ABCG2 alleles.

2. Identification and Genotyping of the Polymorphisms and Statistical Analysis

DNA samples were isolated from blood samples of 50 lactating cows and 5 breeding bulls using a commercial kit (QIAGEN DNA Mini Kit) following manufacturer instruction. 18 set of primers (Table 1) of DGAT1, SCD, and ABCG2 genes were used to amplify all exons (some cases specific exons and introns) and their partial flanking intronic sequences to identify potential SNPs. Primers were designed based on the reference sequence of the bovine *DGAT1, SCD*, and *ABCG2* genes (Gene Bank Accession No. AC000171, AY241932, and AC000163) taken from NCBI and using Primer3 (v.0.4.0.) (http://frodo.wi.mit.edu/) and Net Primer (www.PremierBiosoft.com) web based program and synthesized by Invitrogen Life technologies, Beijing, China. The PCR amplifications of *DGAT1, SCD* and *ABCG2* genes were performed using 18 sets of primers and pooled DNA from 50 lactating

cows and 5 breeding bulls with a programmable thermal cycler. PCR reaction mixture contained 0.2mM dNTPs, 2mM MgCl2, and 0.05U Taq DNA polymerase in final concentration of 20µl volume. The amplification condition was: 5 min at 94ºC for initial denaturing followed by 30 cycles at 94ºC for 30 s; annealing at Tm (ºC) for 30 s,

Table 1. PCR primers for sequencing and SNPs detection

Gene	Exons/introns	Sequence (5' to 3')	Length (bp)
SCD	1	F- GTTGGCAACGAATAAAAGAGG	21
		R- CGCGGTGATCTCAACTCTTC	20
	2	F- GGACCGGGTCTATGCCTATC	20
		R- CCATCCAGCCTCTCAGGAC	19
	3	F- GTTCCCTGGGACTCCTAAGC	20
		R- CCGGAACTTAACCACAAGGA	20
	4	F- GGCAACTCCATGACTTCTCC	20
		R- CATGACCGTCCTAGGTCAAC	20
	5	F- CCCATTCGCTCTTGTTCTGT	20
		R- CGTGGTCTTGCTGTGGACT	19
	6	F- GCCTCTGAGGGGATCTATTTG	21
		R- AGGCAGAGTTGTTGGCTTTC	20
DGAT1	1	F-GGACTACAAAGGTATGCGCG	20
		R-CCGTATCAGGGGTCAAAGGT	20
	2	F-CTTCCGTCTTGCATCACCAG	20
		R-ACAGAGCTCCATTCACCACA	20
	3	F-TCGCAGATCTTAAGCAACGC	20
		R-TTGAGGCAAGTCAGGGGAG	19
	4	F- TTTCGTGGCCTTCCTGAGAG	20
		R-TAGGTCAGGTTGTCGGGGTA	20
	5	F-CTACCCCGACAACCTGACCTA	21
		R- GGGCTTCATGGAGTTCTGGAT	21
	6	F- TCCCCAACCACCTCATCTG	19
		R- GCGGAGGCCAGAAACACT	18
	7	F- CCCCGCAGACACTTCTACAA	20
		R- CAAAGCAGTCCAACACCCAC	20
	8	F-CACCATCCTCTTCCTCAAG	19
		R-AAGGAAGCAAGCGGACAG	18
ABCG2	1	F- TAAAGGCAGGAGTAATAAAG	20
		R- TAACACCAAACTAACCGAAG	20
	2	F- CAGGGCTGTTGGTAAATCTCA	21
		R- GCACGGTGACAGATAAGGAGA	21
	3	F- GGCGTCTGGCTTCAATACTTG	21
		R- ACAGGTGACAGATAAGGAGAACAT	24
	4	F- GTATTCACGAGACTGTCAGGG	21
		R- GGCTTTATTCTGGCTGTTTCC	21

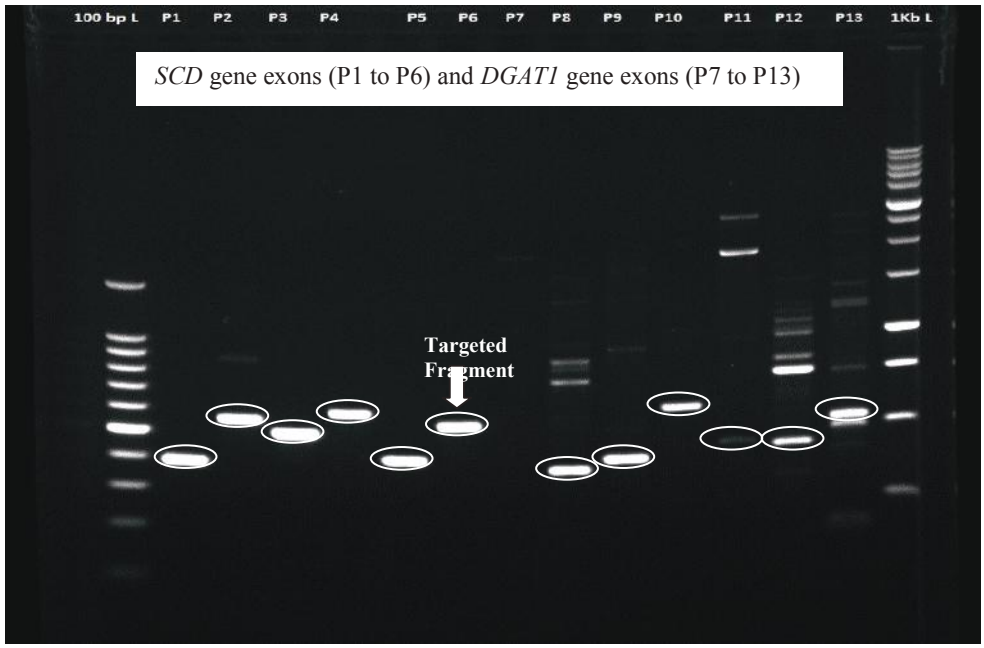


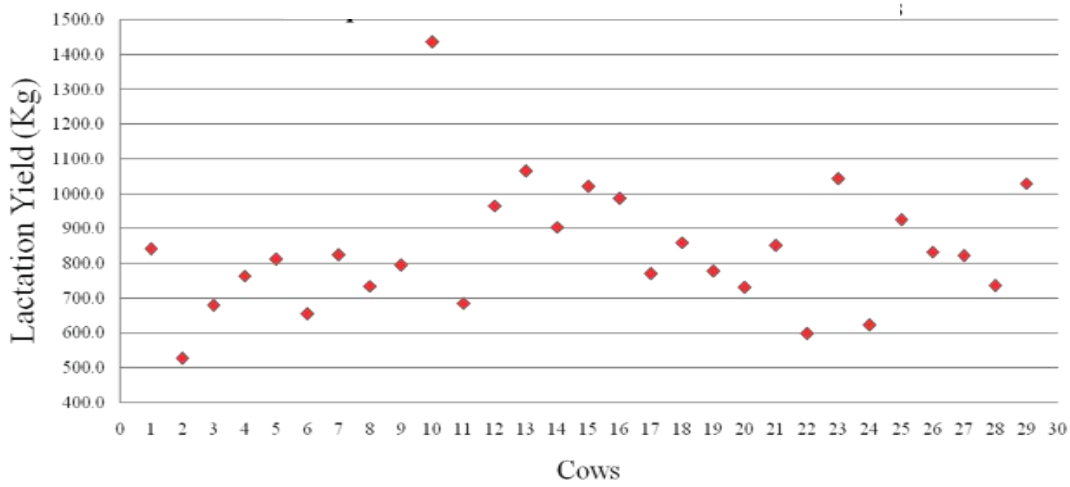
Figure 1. PCR amplification of *SCD* and *DGAT1* Genes

72ºC for 30s; a final extension at 72ºC for 10 min for all primers. Amplification was confirmed by gel electrophoresis of the PCR products in 2% agarose gel followed by visualization under UV (Figure 1).

The targeted DNA fragment, P6 (Figure 1) was cut from agarose gel for DNA isolation. Isolated DNA fragments from agarose gel were used in PCR amplification to get purified PCR products. The purified PCR products were sequenced using Genetic Analyzer ABI 3730 in 1st BASE Laboratory, Malaysia. BioEdit Sequence Alignment Editor, version 7.0.9.0 (Hall 1999) and ClustalW multiple sequence alignment programs (Hall 1999) were used to align both forward and reverse sequences and to determine the presence of SNPs.

Table 2. Phenotypic variation in milk yield, lactation length, fat, protein, lactose and SNF of RCC cows

Parameter	Milk yield (kg)	Lactation length (days)	Fat %	Protein %	Lactose %	SNF %
Mean±SD (n)	838±179(29)	219±35(29)	5.26±0.42 (15)	4.05±0.07 (15)	5.82±0.10 (15)	10.75±0.17 (15)
Maximum	1436	305	6.25	4.13	5.95	10.96
Minimum	527	142	4.71	3.86	5.57	10.27



Graph 1. Location Yield of individual RCC cows

Table 3. Phenotypic variations in lactation yield

Lactation yield (kg)	No. of cows	% C ows
>500	6	20
>700	18	62
>1000	5	18
Total recorded cows	29	100

Results and Discussion

Phenotypic variation within RCC breed in ex-situ condition had been identified based on milk production traits so far lactation yield varied from 527 to 1436 kg per lactation with an average of 838±179(29) kg. The lactation length varied from 142 to 305 days with an average of 219±35 days. There was small variation in milk fat, protein, lactose, and SNF (Table 2) of RCC.The RCC cows were categorized according to its milk yield (Graph 1). About 18% of lactating cows showed an average of >1000 kg milk per lactation and 20% of cows showed >500 kg milk per lactation (Table 3).

The RCC has within breed phenotypic variation in milk yield.No variation was observed in RCC breed for three genes *(SCD, DGAT1* and *ABCG2)* in this study.The studied genes *(SCD, DGAT1,* and *ABCG2)* were fixed in RCC, which are indirectly responsible for higher milk fat yield, higher fat and protein percent. The *SCD, DGAT1* and *ABCG2* allele found fixed in RCC from sequencing results (e.g. Figure 2).The DGAT1 and ABCG2 alleles were also found as fixed in Indian Cattle (Bos indicus) by Tantiaet *et al*.(2006). Cohenetal, 2005 reported that an increase in frequency of ABCG2Y allele with selection for higher milk fatand protein percentage in the Israeli Holstein population. The phenotypic variations in milk production traits, which, found within RCC breed at

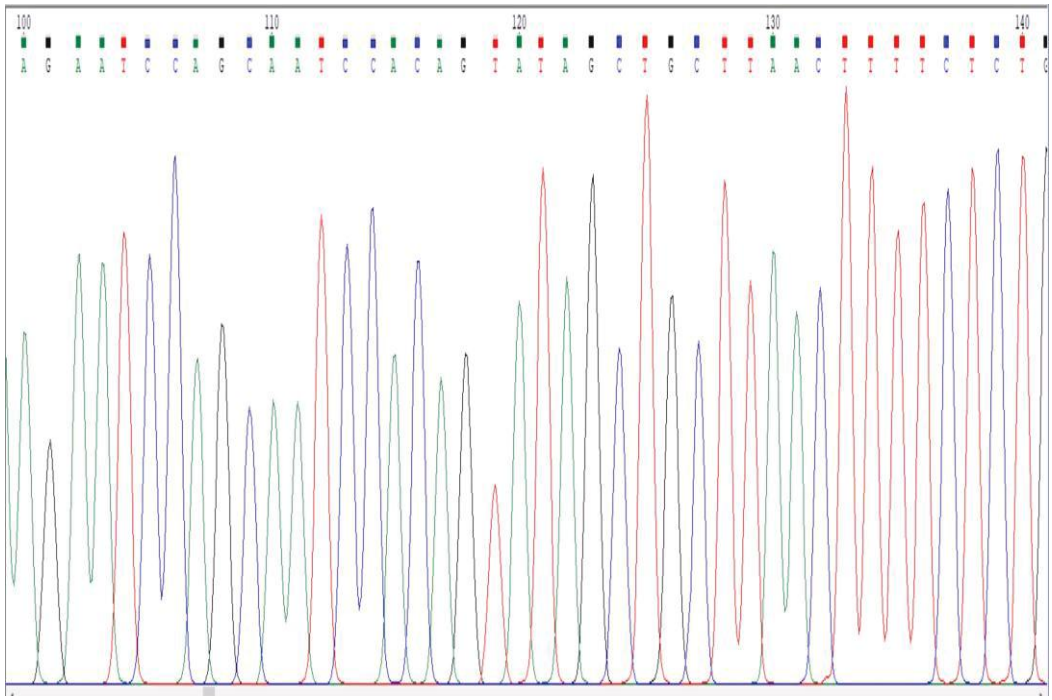


Figure 2. Chromatograph of SCD gene indicating no SNPs in exon 6

BLRI herd were not due to the effect of *SCD, DGAT1* and *ABCG2* genes. Besides these 3 genes, there are many other genes involved in milk production and metabolism pathways like *FASN, CSN3, PPARGC1A, IGF1, OLR1, PRL, STAT5A, GHR* (Mele *et al*,. 2007, Moioli *et al.*, 2007, Schennink *et al.*, 2008, Schennink *et al.*, 2009, Sun *et al.*, 2009), which might have effect on the studied phenotypic variation.

Conclusion

This study provides indirect evidence that RCC breed has fixed alleles with respect to *SCD, DGAT1* and *ABCG2* genes reported to be responsible for higher milk fat yield, higher fat and protein percent. There is considerable genetic variation in milk yield in RCC which can be utilized to increase the milk yield without having an adverse effecton fat and protein percent. However, further study needed to unlock the genetic reason behind these phenotypic variations using other genes involved in milk production and metabolism pathways.

References

Alim, M.A., Fan, Y.P., Wu, X.P., Xie, Y., Zhang, Y., Zhang, S.L., Sun, D.X., Zhang, Y., Zhang, Q., Liu, L., Guo, G. 2012. Genetic effects of stearoyl-coenzyme A desaturase (SCD) polymorphism on milk production traits in the Chinese dairy population. Molecular Biol. Report 39: 8733-8740.

Boichard, D., Fritz, S., Rossignol, M.N., Guillaume, F., Colleau, J.J., Druet, T. 2006. Implementation of marker-assisted selection: practical lessons from dairy cattle. 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, MG, Brasil. August 13-18.

Cohen, M., Seroussi, E., Larkin, D. M., Loor, J.J., Everts-van der Wind, A., Heon-Lee, J.,

Drackley, J.K., Band, M.R., Hernandez, A.G., Shani, M., Lewin, H.A., Weller, J.I., Ron, M. 2005. Identification of a missense mutation inthe bovine ABCG2 gene with a major effect on the QTL onchromosome 6 affecting milk yield and composition in Holstein cattle. Genome Res. 15: 936-944.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT Nucleic Acids Symp. 41: 95-98.

Mele, M., Conte, G., Castiglioni, B., Chessa, S.; Macciotta, N.P., Serra, A., Buccioni, A., Pagnacco, G., Secchiari, P. 2007. Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy Sci. 90: 4458-65.

Moioli, B., Contarini, G., Avalli, A., Catillo, G., Orru, L., De-Matteis, G., Masoero, G., Napolitano, F. 2007. Short communication: Effect of stearoyl-coenzyme A desaturase polymorphism on fatty acid composition of milk. J. Dairy Sci. 90 : 3553-8.

Ruane, J. and Colleau, J.J. 1996. Marker assisted selection for a sex-limited character in a nucleus breeding population. J. Dairy Sci. 79: 1666-1678.

Schennink, A., Bovenhuis, H., Leon-Kloosterziel, K.M., Van Arendonk, J.A.M., Visker, M. 2009. Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. Animal Genetics. 40: 909-16.

Schennink, A., Heck, J.M., Bovenhuis, H., Visker, M.H.,Van Valenberg, H.J.,Van Arendonk, J.A. 2008. Milk fatty acid unsaturation: genetic parameters and effects of stearoyl-CoA desaturase (SCD1) and acyl CoA: diacylglycerolacyltransferase 1 (DGAT1). J. Dairy Sci. 91: 2135-43.

Sun, D., Jia, J., Ma, Y., Zhang, Y., Wang, Y., Yu, Y. 2009. Effects of DGAT1 and GHR on milk yield and milk composition in the Chinese dairy population. Animal Genetics. 40: 997-1000.

Tantia, M. S., Vijh, R.K., Mishra, B.P., Bina, M., Kumar, B.S.T., Sodhi, M. 2006. DGAT1 and ABCG2 polymorphism in Indian cattle (Bos indicus) and buffalo (Bubalus bubalis) breeds, BMC Vet. Res. 2: 32.