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Association of DGAT1, beta-casein and leptin gene polymorphism with milk quality and yield traits in Jersey and its cross with local Kashmiri cattle

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Abstract

This study investigated the impact and significance of polymorphisms in DGAT1, beta-casein and Leptin gene on milk quality and yield traits in Jersey and its local Kashmiri cross. A total of 200 animals were phenotyped for milk yield, fat content, protein content, fat yield and protein yield for a period of 305 days (January-October). The genotyping of animals for SNPs was done through PCR-Sequencing method. Three genotypes with respect to DGAT1-K232A polymorphism (KK, KA and AA) and only two genotypes of beta-casein (A1A2, A2A2) and leptin (CC, CT) gene were found in the studied population. In the association study K allele of DGAT1-K232A polymorphism was significantly associated with fat content, protein content and fat yield, whereas, A allele was correlated with protein yield and milk yield. A2 allele of beta-casein gene was found significantly associated with fat and protein content and A1 allele showed correlation with high protein and milk yield. On the other hand, C allele of leptin gene shows significant association with fat content only. The study suggests that the SNPs analyzed in this study associated with different milk traits and hence could be used as genetic markers for selection of dairy animals in breeding programs.

Keywords: single nucleotide polymorphism, allele, milk production traits, MAS

Introduction

Genetic research in farm animals focuses mainly on the identification of genes influencing economically important traits that could be useful in breeding programs [1]. Given the fast pace of development of the genetic markers for quantitative traits, it would be worthwhile to make a beginning now to supplement the selection procedures based on phenotypic data with marker assisted selection (MAS) [2]. This kind of selection is based on the identification of genes or markers that may affect economically important traits of dairy cows. Several polymorphisms in various gene loci have been reported to affect production traits such as milk yield and composition. In dairy cattle, most studies have researched the genes that may determine variations in milk yield and milk composition [3]. A number of candidate genes have been reported that affect the yield and composition of milk in cattle viz. beta-Casein, β -lactoglobulin [4], FMO3 [5], DGAT [6], BGRH [7], leptin gene [8].

In the current study we investigated the effect of gene polymorphism of candidate genes (DGAT1, beta-casein and Leptin) on milk production and composition traits. DGAT1 is a candidate gene related to variation in milk fat content in dairy cattle. This gene is localized on the centromeric end of the 14th bovine chromosome. A base substitution (K232A) in the *DGAT1* gene in exon number 8 led to the quantitative trait loci variation [6]. While the lysine variant (K allele) of the *DGAT1* gene was associated with high fat content of the milk, the alanine variant (A allele) of the *DGAT1* gene was associated with high milk yield. The lysine encoding variant is considered to be the ancestral state of *DGAT1*, and mutation is believed to have taken place early in the history of the domestication of cattle or even before domestication [6, 9]. Beta-casein is a member of casein cluster and with 13 protein variants known it is the most polymorphic milk protein gene. The variants most common in different cattle breeds are A1 and A2, the others (e.g. B, A3, C) being rare [10]. Polymorphism in one of the beta-casein gene codons – CCT → CAT – causes a substitution of proline (A2) by histidine (A1) in position 67 in the amino-acid sequence.

That polymorphism was reported to increase protein yield and milk yield as well as to decrease milk fat content and yield^[11, 12, 13]. Leptin (LEP), a 4067 bp gene located on chromosome 4 consisting of three exons and two introns^[14]. This gene plays a role in regulating body weight and reproductive function^[15], growth of breast cells and milk secretion^[16]. In exon 3 of this gene, Buchanan *et al.*^[17] found a C/T transition leading to the substitution of alanine to valine (A59V) and this tended to change the milk production traits. It was found by Banos *et al.*^[18] that T variant was associated with less fat content, protein content and milk yield; whereas, C allele shows correlation with different milk quality traits. Considering the DGAT1, beta-casein and Leptin as strong candidates for gene assisted selection for milk yield and fat content, the present study was aimed to study their polymorphisms and association with milk production traits in Jersey cattle and its cross with local Kashmiri cattle.

Material and Methods

Animals and Phenotyping

The experiment was conducted on Jersey and its cross with local Kashmiri cattle taken from university Dairy cattle farm, Srinagar (Jammu and Kashmir). Total of 200 cows were selected for the current study and selection of animals was based on parity, age and health status of animals. The milch animals in 3rd and 4th lactation having good health condition were identified and were subsequently phenotyped for milk quality and yield for full lactation (January-October). Milk yield of the cows was recorded for a full lactation period of 305-days. Milk samples were collected weekly from each cow and were analyzed for different quality parameters like protein percentage and fat percentage. Fat yield and protein yield were calculated mathematically from their respective percentages.

DNA Extraction

Blood samples were collected from all the 200 cows aseptically from jugular vein in a tube treated with EDTA. All the samples were delivered back to the laboratory in an ice box. The genomic DNA was collected from whole blood using standard phenol/chloroform extraction protocol^[19].

Genotyping

The alleles and genotypes of the three genes were analyzed by polymerase chain reaction (PCR) followed by sequencing. Primers for the PCR (Table1) were designed, using primer3+ based on gene sequences available in the GenBank database (DGAT1 - JQ897353, Beta-Casein - JN559864.1, Leptin-AJ512). PCR amplification was performed in a thermo cycler (Applied Biosystem). The reaction mixture contained in 25 μ l a total of 25-50 ng genomic DNA, 0.05 unit of *Taq* polymerase, 1x PCR buffer, 1.5Mm MgCl₂ (For DGAT1 5% DMSO was added), 0.5pM of each primer. Thermal cycling

conditions were as follows: initial denaturation of 5 min. (15 min for DGAT1 gene), cyclic denaturation at 94°C for 30 sec, annealing temperature (table2) for 60 sec and extension at 72°C for 60 sec, followed by final extension for 10 min. at 72°C. The amplified products were sequenced commercially (Macrogen Inc. Korea). The sequenced data was analyzed for detection of SNPs through SNAP-Gene viewer software.

Statistical Analysis

The allelic frequencies and heterozygosity were calculated using Genepop software (version 4.0). The software SPSS (version 20.0) was used to analyze the relationship between genotypes and milk yield, fat content, protein content, fat yield and protein yield.

Results and Discussion

Polymorphisms of DGAT1, beta-casein and Leptin gene

The sequencing results (Fig. a, b, c) of the amplified products of three different genes were analyzed for identification of SNPs in Jersey and its cross. The frequency of different alleles and genotypes are shown in Table 2. The frequency of K and A allele for DGAT1-K232A polymorphism was found to be 0.6 and 0.4 for Jersey, respectively, which are in consistent with the findings of Komisarek *et al.*^[20], whereas the frequency of K and A allele were found to be 0.58 and 0.42, respectively in Jersey cross. The relative frequency of different genotypes KK, KA and AA were found to be as 0.43, 0.33 and 0.23 for Jersey and 0.43, 0.30 and 0.27 for its cross.

For beta-casein gene the frequency of A1 and A2 allele in Jersey breed were found to be 0.25 and 0.75, respectively. As reported by Kaminski *et al.*^[21] frequency of A1 allele varies from 0.09-0.26, hence our results are inconsistent with previous findings. Besides health promoting properties, A2 variant has also been reported to have position relationship with milk performance traits as reported by Freyer *et al.*^[22]. This may be the reason of high frequency of A2 allele in comparison to A1 allele. The frequency of A1 and A2 allele in Jersey cross has been found as 0.057 and 0.942, respectively. The same allele frequencies in Indian zebu cattle were reported by Indrajit *et al.*^[23] the corresponding genotype frequencies of A1A2 and A2A2 genotypes in Jersey were found as 0.5 and 0.5, which are in good agreement with the previous findings, whereas in cross the corresponding were found to be 0.1140 and 0.885. At the leptin- A59V locus, the frequency of C and T allele in Jersey was found to be 0.775 and 0.225. Whereas, in jersey cross the corresponding frequencies were recorded as 0.829 and 0.171. The present data were consistent with those shown by Kuling *et al.*^[24] on Jersey breed. There was no animal with TT genotype in either of the breed, which are in good agreement with the findings of Madeja *et al.*^[25], Liefers *et al.*^[26].

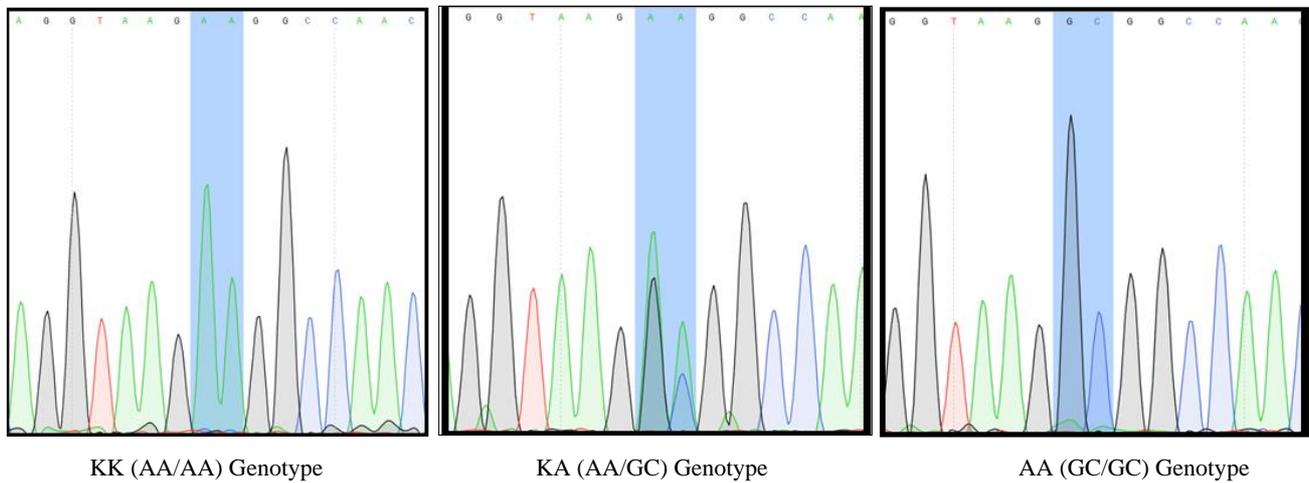


Fig a: Partial sequence maps of different genotypes of DGAT1 gene

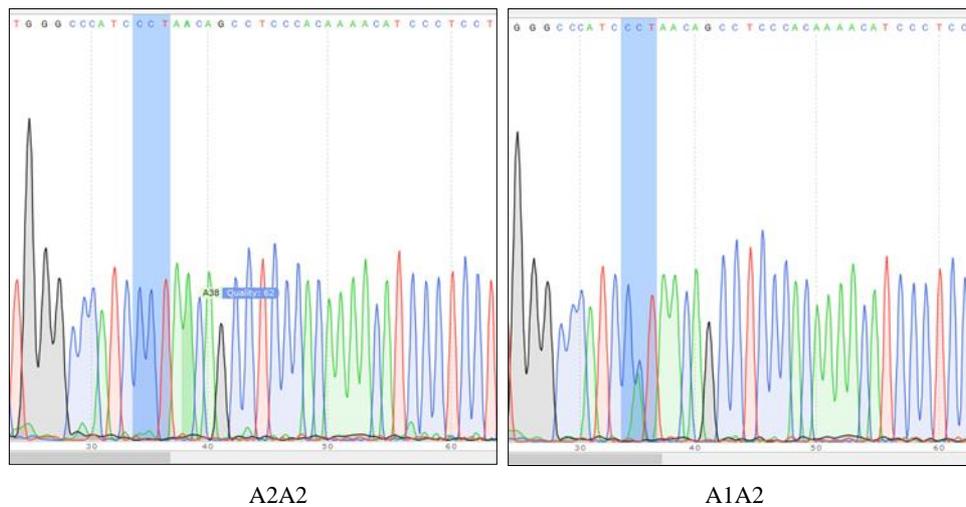


Fig b: Partial sequence maps of different genotypes of beta-casein gene

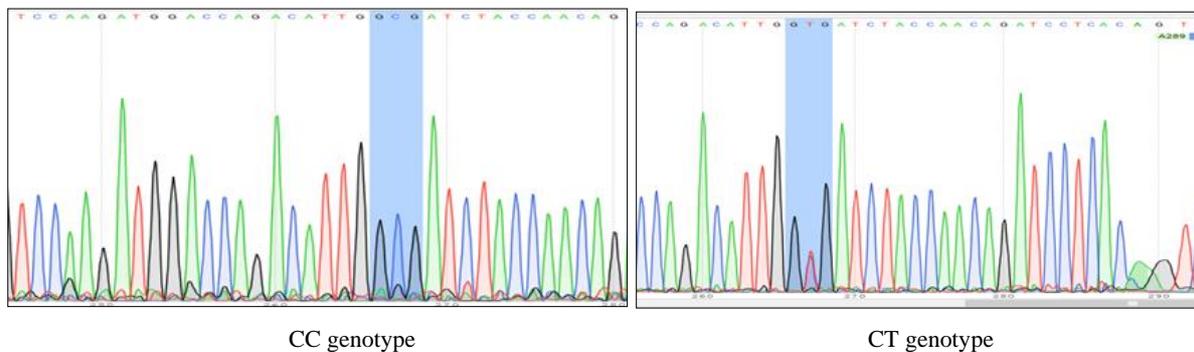


Fig c: Partial sequence maps of different genotypes of leptin gene

Association of DGAT1, beta casein and leptin gene polymorphism with milk production traits

The KK homozygotes of DGAT1 gene K232A polymorphism were characterized by highest average fat content, protein content and fat yield that was significantly ($P < 0.05$) higher than individuals with KA and AA genotypes (Table 3). Positive effect of allele K on fat content and protein content was also found in earlier studies however AA genotype was found to be associated with higher milk yield and protein yield. Such findings are also reported by Berey *et al.* [27]. Effects of DGAT1 gene K232A polymorphism on milk production traits in Jersey cow population were estimated earlier by Komisarek *et al.* [20], which are in consistent with the findings of our study. The same results were also reported by Grisart *et al.* [6] and Kupe *et al.* [28] who studied different

cattle breeds. The evolutionary conserved lysine residue at position 232 may be important for the interaction of the CoA moiety with DGAT enzyme. Hence alanine residue at position 232 could have a negative effect on the acyl-CoA-binding capacity of DGAT, accounting for its associated lower milk fat content. The lysine variant represents more efficient allele with regard to fat synthesis, because it is present at a high frequency or exclusively in species and breeds with a higher milk fat content an in animals from the high end of the trait distribution, as also confirmed by our findings.

For Beta casein A1/A2 polymorphism, it was found that individuals with A2A2 genotype were associated with high fat and protein content ($P < 0.05$), whereas A1A2 individuals were associated with high milk protein yield.

To date there are no published research focused on detailed

observations of leptin gene A59V polymorphism effect on milk production traits. Detailed observation of milk production parameters for a full lactation period of 305 days (Table 3) showed significantly ($P<0.05$) higher fat content in

CC genotype individuals [26]. Any significant differences in other parameters were not found, only tendency of CC genotype to increase milk and protein content.

Table 1: Selected PCR conditions for the polymorphisms considered in this study.

SNP	Primers	Annealing temperature	PCR Product size	Gene region
DGAT1-K232A	F-GCACCATCCTCTTCCTCAAG R-GGAAGCGCTTTCGGATG	60°C	411	Exon 8
Beta-casein-A1A2	F-CCAGGATAAAATCCACCCCT R-AGGGAAGGGCATTTCCTTGT	58°C	202	Exon 7
Leptin -A59V	F-GGGAAGGGCAGAAAGATAG R-TGGCAGACTGTTGAGGATC	59°C	330	Exon3

Table 2: Frequency of allele and genotype of DGAT1, beta-casein and Leptin gene in Jersey and its Local Kashmiri cross.

Gene	Breed	Genotype	Genotype frequency	Allele Frequency	
DGAT1	Jersey	KK	0.43	0.6	0.4
		KA	0.33		
		AA	0.23		
	Cross	KK	0.43	0.58	0.42
		KA	0.30		
		AA	0.27		
CSN2	Jersey	A1A2	0.5	0.25	0.75
		A2A2	0.5		
		A1A1	0		
	Cross	A1A2	0.114	0.057	0.942
		A2A2	0.885		
		A1A1	0		
Leptin	Jersey	CC	0.55	0.775	0.225
		CT	0.45		
		TT	0		
	Cross	CC	0.657	0.829	0.171
		CT	0.342		
		TT	0		

Table 3: DGAT1, beta-casein and Leptin gene polymorphism and their association with milk quality and yield traits

Gene	Breed	Genotype	Fat%	Fat yield	Protein%	Protein yield	Milk yield
DGAT1	Jersey	KK	5.72 ^a ±0.08	79.86 ^a ±6.85	3.52 ^a ±0.05	42.22 ^a ±3.74	1594 ±101.2
		KA	4.95 ^b ±0.13	64.06 ^b ±1.56	3.31 ^b ±0.07	54.72 ^a ±3.12	1734 ±182.08
		AA	3.70 ^c ±0.13	60.04 ^b ±1.7	3.26 ^{ab} ±0.12	64.24 ^{ba} ±6.32	1954 ±49.05
	Cross	KK	4.64 ^a ±0.13	51.14 ^a ±3.14	3.41 ^a ±0.04	38.9 ±1.28	1140.6 ±56.46
		KA	4.25 ^b ±0.17	49.8 ^a ±5.22	3.26 ^a ±0.07	40.8 ±0.96	1247.24±53.56
		AA	2.63 ^c ±0.08	35.94 ^b ±2.58	3.06 ^b ±0.01	41.38 ±1.76	1352.6± 81.76
CSN2	Jersey	A1A2	5.01 ^b ±0.41	68.10 ±4.97	3.49 ^b ±0.47	52.43 ±4.12	1833.5 ± 47.05
		A2A2	5.75 ^a ±0.78	76.63 ±5.99	3.61 ^a ±0.09	47.35 ±3.77	1635.40±37.88
	Cross	A1A2	3.53 ±0.34	39.32 ±3.89	3.01±0.8	37.80 ±1.32	1203.61±56.10
		A2A2	4.10 ±0.29	47.67 ±4.12	3.36±0.32	32.43 ±1.99	1045.19±48.30
Leptin	Jersey	CC	5.53 ^a ±0.09	104.35±5.89	3.63 ±0.09	68.49 ±2.43	1887 ±89.56
		CT	4.78 ^b ±0.19	84.36 ±6.11	3.21 ±0.13	56.65 ±1.96	1765 ±105.3
	Cross	CC	4.5 ^a ±0.12	59.58 ±3.98	3.39 ±0.32	44.88 ±3.32	1324 ±75.10
		CT	3.87 ^b ±0.17	49.03 ±5.34	2.98 ±0.67	37.75 ±1.34	1267 ±85.32

Figures quoted as mean and standard deviation.

Figures having different superscripts within the column vary significantly ($p<0.05$)

Conclusion

It is evident from the results of the present study that the different allelic variants of DGAT1, leptin and beta-casein gene are associated with various milk production traits. The different allele and genotype frequencies in the studied population indicate the genetic variability within the population, hence these polymorphisms can be used as genetic markers in marker assisted selection programs for the improvement of milk production traits in dairy cattle. However, a robust candidate gene approach is required to develop a molecular marker score with respect to milk traits and to frame an integrated breeding policy for faster genetic improvement of dairy animals.

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