



Single nucleotide polymorphism (g.2786 A>G) of *DGAT1* gene associated with milk yield and fat percentage in crossbred cattle

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ABSTRACT

In present study, association of five single nucleotide polymorphisms (SNPs) of database rs109421300, rs43691049, rs109727821, rs109047657 and rs135678421 was investigated with milk minerals including calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), sodium (Na), potassium (K) and constituents (fat and protein percentage) after adjusting the records with various significant non-genetic factors in crossbred (*Vrindavani*) cattle. The effect of rs109421300 pertaining to Diacylglycerol Acyltransferase (*DGAT*) gene located on BTA 14, was highly significant on fat percentage and significant on test day milk yield and non-significant on Ca, Cu, Mn, Zn, Fe, Na, K, test day milk yield and protein percentage. The AA genotypes of rs109421300 locus had significantly lower (2.96±0.17%) fat percentage than AG (4.64±0.22%), GG (4.62±0.27%) genotypes but simultaneously cows with AA genotypes had significantly highest (11.59±0.51 kg) test day milk yield than other two genotypes. At locus rs109727821, the manganese concentration was significantly highest (1.64±0.16 mg/l) for AG followed by AA (0.98±0.15 mg/l) and GG (0.77±0.24 mg/l) genotypes. The other SNPs had no significant association with traits under investigation. The existing association suggested possibilities to select animals for specific minerals and constituent traits in crossbred population.

Key words: Cattle, *DGAT1*, Milk minerals, Milk fat, Single nucleotide polymorphism

Bovine milk provides important minerals and constituents, essential for human nutrition and dairy product quality. In recent decades, interest has been growing in the quality and technological properties of milk. In the dairy industry, contemporary breeding goals have expanded to include, along with milk production characteristics, health and functional traits in an effort to improve the overall functionality of the dairy cattle (Viale *et al.* 2017). Besides water, milk consists of proteins, fat, lactose, vitamins and minerals, in addition to other components like metabolites and free oligosaccharides. Milk fat is the main source of energy and natural carriers of triacylglycerols and bioactive molecules (polar lipids and proteins) to the intestine of mammalian infants. Milk contains a wide array of proteins with biological activities ranging from antimicrobial ones to those facilitating absorption of nutrients, as well as acting as growth factors, hormones, enzymes, antibodies and

immune stimulants (Korhonen *et al.* 1998). Dairy products are considered as the primary source of calcium and phosphorus, as well as significant sources of sodium, potassium, zinc, manganese and iron (Helland-Kigen 2015). Milk mineral contribute to several vital physiological processes for example, Ca and P play an important role in bone metabolism, Zn in immune responses, and Ca, K, and Mg in the regulation of blood pressure (Cashman 2006, Haug *et al.* 2007). Genetic variability in the relative proportions of the various milk constituent and mineral traits indicates the possibility of using genomic selection to improve milk traits (Bilal *et al.* 2012). Sequencing and genotyping technologies in the last few decades, made it possible to scan the genome of domestic animals for thousands of single nucleotide polymorphisms to capture the genetic variants responsible for economically important traits (Daw *et al.* 2005). Therefore, the aim of this study was to find association of single nucleotide polymorphism with milk constituent and mineral traits.

MATERIALS AND METHODS

Experimental animals and farm: A total of 120 lactating crossbred (*Vrindavani*) cattle studied in this research were taken from Cattle and Buffalo farm of the Indian Veterinary Research Institute, Izatnagar. The Vrindavani cattle, a synthetic crossbred strain of cattle, was developed using

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Table 1. SNPs information with reference to traits under investigation

SNPs	Trait (Reference)	Sequence (5'-3')	Bases	Target regions	Amplicon size (bp)	Annealing temperature
rs109421300	Calcium, Copper, Zinc, Phosphorus and Fat* (Jiang <i>et al.</i> 2010)	F GGCAGGCTTGGACTTCACT R GAAATAACCGTGCGTTGCTT	19 20	Chromosome: 14:1801116	359	54°C
rs43691049	Milk yield, Fat yield, Protein yield* (Jiang <i>et al.</i> 2010)	F GGGTCAGAGTGCAGGAGAGA R CGTTCTCCCTGAAAGACAGC	20 20	Chromosome: 11:103303343	333	55°C
rs135678421	Manganese* (Buitenhuis <i>et al.</i> 2015)	F TGAAGGGTGAAGCAAGTA R TTGAGGGCTCCATTACAGT	20 20	Chromosome: 5:114872802	381	59°C
rs109047657	Sodium* (Buitenhuis <i>et al.</i> 2015)	F TAAATGGTCATCTGGCAGCA R GGGGAAACGGCTATAGGAAG	20 20	Chromosome: 9:26623724	333	57°C
rs109727821	Potassium* (Buitenhuis <i>et al.</i> 2015)	F AGATTGGGATGCGTTGTTC R AGCAGCTGGAACCTCCGTAGA	20 20	Chromosome: 28:45822795	343	59°C

Top 20 and bottom 20 cows against each SNP were selected on the basis of the average of the trait marked with '*' as above.

Holstein-Friesian, Brown Swiss, Jersey and Harijana breeds (Singh *et al.* 2011). Animals were maintained under loose housing system and nutritional requirements of animals were fulfilled through a balanced combination of dry and green fodder with concentrate mixture supplementation. Milk recording was done from the first day of calving till date of drying and calves weaned immediately after birth.

Milk samples and chemical analysis: Approximately 40 ml of fresh milk samples were collected twice from each animal, first in the month of December and second in the month of March. Before collection of the milk sample, it was ensured that it should be thoroughly mixed. Total fat and protein percentage was determined by using Lactoscan. Calcium and phosphorus estimation in milk samples were done by Talapatra method (Talapatra *et al.* 1940) and Gravimetric method, respectively. The Na and K estimation was done by flame photometer, whereas, trace minerals (Zn, Cu, Fe and Mn) in different milk samples were estimated by atomic absorbance spectrophotometer in their respective mineral extracts.

DNA extraction and genotyping: Blood samples were collected from 120 cows and DNA extraction was carried out using the phenol-chloroform extraction method. Based on the earlier reports, SNPs rs109421300 (for fat percentage), rs43691049 (for protein percentage), rs109727821 (for manganese), rs109047657 (for sodium) and rs13567842 (for potassium) were selected in present study. *In silico* primer designing for target region of SNPs was carried out using PRIMER 3 software as shown in Table 1. The top 20 and bottom 20 animals for targeted milk mineral/constituent against each SNP were selected based on average of two month (March and December). The genomic DNA samples were amplified by PCR and amplified PCR products of both sets of primers were outsourced for purification and custom sequencing from

both ends (5' and 3' ends) using Sanger sequencing.

Statistical analysis: The influence of non-genetic factors (parity, lactation stage, test day milk yield and month of collection etc.) on different milk constituent traits was studied by least squares analysis method (Harvey 1990) for non-orthogonal data using following model

$$Y_{ijkln} = m + P_i + L_j + T_k + M_l + e_{ijkln}$$

where Y_{ijkln} is the n^{th} observation (traits) of i^{th} parity, j^{th} lactation stage, k^{th} test day milk yield, l^{th} month of milk collection; P_i is effect of i^{th} parity ($i=1$ to 3); L_j is effect of j^{th} month of milk collection ($j=1$ to 3); T_k is effect of k^{th} test day milk yield ($k=1$ to 3); M_l is month of milk collection ($l=1$ to 2) and e_{ijkln} is random error ($NID \sim 0, \sigma_e^2$). For all milk minerals (except Na and K), fat percentage and protein percentage, the above mentioned mixed model was used. However, since Na and K content of milk was estimated only once in month of December, hence effect of month of collection was removed from model for analyzing the Na and K data.

In order to analyze, the association of each SNP to the milk constituent and mineral traits, the following statistical model was used:

$$Y_{ij} = m + X_i + e_{ij}$$

where Y_{ij} , j^{th} observation of milk minerals and constituents traits; X_i , effect of i^{th} genotype and e_{ij} , random error

RESULTS AND DISCUSSION

The mean (average) calcium, phosphorus, iron, copper, manganese, zinc, sodium and potassium concentration in crossbred ($n=120$) milk was 1446 ± 0.03 mg/l, 1268 ± 0.02 mg/l, 9.67 ± 0.22 mg/l, 1.10 ± 0.04 mg/l, 1.10 ± 0.04 mg/l, 3.76 ± 0.10 mg/l, 21.40 ± 0.44 meq/l and 33.53 ± 0.60 meq/l, respectively. The estimated mean fat and protein percentage

Table 2. The least squares mean of milk minerals and constituents for genotypes of different SNPs

SNPs	Genotype	N	P (%)	Ca (%)	Cu (mg/l)	Mn (mg/l)	Zn (mg/l)	Fat (%)	Protein (%)	Fe (mg/l)	TDY (kg)
rs109421300	AA	16	1.37±0.09	1.59±0.13	1.40±0.30	1.51±0.19	3.56±0.32	2.96 ^a ±0.17	2.76±0.05	9.57±0.75	11.59 ^a ±0.51
	AG	40	1.28±0.06	1.53±0.10	1.06±0.14	1.37±0.14	4.06±0.31	4.64 ^b ±0.22	2.78±0.03	10.03±0.75	10.85 ^{ab} ±0.44
	GG	24	1.21±0.10	1.37±0.09	1.03±0.20	0.99±0.18	3.91±0.41	4.62 ^b ±0.27	2.82±0.02	11.15±0.8	9.77 ^b ±0.44
rs43691049	AA	4	1.28±0.29	2.01 ^a ±0.12	0.84±0.41	0.91±0.44	2.85±0.91	4.27±0.60	2.95±0.08	10.02±2.01	9.82±1.66
	AG	24	1.42±0.10	1.45 ^b ±0.08	1.09±0.18	1.31±0.18	3.61±0.40	6.05±1.48	2.81±0.04	10.95±0.80	10.82±0.55
	GG	52	1.34±0.06	1.46 ^b ±0.08	1.31±0.16	1.07±0.12	4.26±0.66	4.17±0.15	2.8±0.04	9.06±0.42	10.38±0.33
rs109727821	AA	36	1.3±0.08	1.380.08	1.18±0.14	0.98 ^a ±0.15	3.95±0.25	4.27±0.17	2.79±0.03	10.47±0.72	10.65±0.40
	AG	32	1.34±0.05	1.40.09	1.09±0.08	1.64 ^b ±0.16	3.97±0.31	4.5±0.22	2.82±0.02	10.22±0.56	11.25±0.43
	GG	12	1.19±0.12	1.410.11	0.86±0.16	0.77 ^a ±0.24	3.79±0.35	4.81±0.29	2.79±0.05	9.53±0.9	10.5±0.81
rs109047657	GG	64	1.24±0.04	1.41±0.07	1.09±0.08	1.26±0.09	3.66±0.20	4.46 ^a ±0.14	2.8±0.02	9.13±0.37	11.23±0.31
	GA	16	1.25±0.07	1.32±0.09	1.16±0.16	1.25±0.17	3.73±0.42	3.89 ^b ±0.16	2.75±0.04	9.67±1.20	11.72±0.57
	GG	6	1.4±0.09	1.64±0.33	1.75±0.68	1.83±0.25	4.88±0.90	4.1±0.43	2.71±0.10	10.84±1.97	12.25±1.11
rs135678421	GT	40	1.28±0.05	1.51±0.08	1.20±0.15	1.31±0.13	3.62±0.26	4.18±0.15	2.83±0.03	8.98±0.56	11.95±0.37
	TT	34	1.34±0.07	1.38±0.08	0.98±0.14	1.24±0.16	3.96±0.36	4.14±0.19	2.83±0.05	10.69±0.76	10.99±0.42

The least squares means with same superscripts do not differ significantly.

was 4.28±0.07 and 2.81±0.01%, respectively. In Holstein-Friesian cows, mean concentration of Ca was reported as 1171 mg/l (Toffanin *et al.* 2015). Zamberlin *et al.* (2012) reviewed slightly lower range of calcium varied from 107 to 133 mg/100 g in cow milk. Various workers reported the range of phosphorus from 825 to 1995 mg/l (Zamberlin *et al.* 2012, Gaucheron 2005, Barlowska *et al.* 2006, Pereira 2014). Zamberlin *et al.* (2012) reviewed the range of iron from 30 to 70 µg/100g in cow milk. In the previous studies, a low range (0.034 to 0.164 mg/l) of the copper was reported in fresh cow milk (Rodríguez *et al.* 2001). However, higher range of Cu from 2 to 30 µg/100g was also reported by several workers in the milk of cattle (Zamberlin *et al.* 2012). The estimates of manganese were slightly variable from 22 µg/l (Abollino *et al.* 1998) and 53±4 µg/l in fresh cow milk (Abollino *et al.* 1998, Martino *et al.* 2001). Other workers (Lavi and Alfassi 1990, Martino *et al.* 2001) had also reported the similar zinc concentrations in the milk of cattle (3.4 mg/l to 3.8 mg/l). However, others have reported lower concentration of zinc in fresh cow milk (4.409±0.666 mg/l) and in sterilized cow milk (3.060±0.137 mg/l) (Rodríguez *et al.* 2001). Similar variability of sodium from 278 to 870 mg/l was found in fresh cow's milk from Canary Island (Rodríguez *et al.* 2001), whereas other worker (Zamberlin *et al.* 2012) reviewed lower range of sodium from 40 to 58 mg/100g in cow milk. Similar concentration of potassium 1424±200 mg/l was found in fresh cow's milk from Canary Island (Rodríguez *et al.* 2001). However, lower range of potassium was reviewed from 144 to 178 mg/100g in cow milk (Zamberlin *et al.* 2012). Similar findings of fat percentage (4.02±0.02% and 3.91±0.14%, respectively) were also reported in the milk of Karan-Fries cattle (Mishra and Joshi 2009, Sarkar *et al.* 2006). Similar mean of protein percentage (3.04±0.03%) was reported in Asom native cattle breed (Kayastha *et al.* 2008). However, higher mean of 3.92±0.05% and 3.60±0.05% was reported in Tharparkar cattle and Sahiwal cattle respectively (Buitenhuis *et al.* 2014). Similarly, several other workers also reported protein percentage of 3.35±0.03% and 3.58±0.04% in Karan-Fries cattle (Mishra and Joshi 2009, Sarkar *et al.* 2006).

The non-genetic factors (parity, stage of lactation, level of test day milk production and month of milk sample collection) significantly affecting the milk mineral and their constituents were adjusted using least squares constants. However, after adjusting the record, associations of these SNPs for different sets of animals were established with all the traits under investigation.

Association of rs109421300 with milk mineral/constituent traits: The allelic frequency of A and G alleles was 0.450 and 0.550 and the genotypic frequencies of AA, AG and GG genotypes were 0.20 (8 animals), 0.50 (20 animals) and 0.30 (12 animals) respectively (Supplementary Fig. 1). The association of genotype was established with adjusted records for the cattle selected for rs109421300. The fat percentage was significantly (P≤0.001) highest (4.64±0.22%) for AG genotype and lowest (2.96±0.17%)

Table 3. The least squares mean of sodium (Na) and potassium (K) for genotypes of different SNPs

SNPs	rs109421300		rs43691049		rs109727821		rs109047657		SNP	rs135678421	
	Na (meq/l)	K (meq/l)	Na (meq/l)	K (meq/l)	Na (meq/l)	K (meq/l)	Na (meq/l)	K (meq/l)		Genotype	Na (meq/l)
AA (n)	20±1 (8)	32±2 (8)	20±4 (2)	34±1 (2)	21±1 (18)	30 ^a ±1 (18)	–	–	GG (n)	19±2 (18)	33±4 (18)
AG (n)	20±1 (20)	35±1 (20)	21±1 (12)	34±1 (12)	23±1 (16)	33 ^{ab} ±1 (16)	22.75±3.19 (8)	32.73±1.90 (8)	GT (n)	22±1 (19)	35±2 (19)
GG (n)	23±1 (12)	33±1 (12)	21±1 (26)	38±2 (26)	21±1 (6)	36 ^b ±4 (6)	23.15±2.02 (32)	33.58±1.19 (32)	TT (n)	20±1 (3)	35±2 (3)

The least squares means with same superscripts do not differ significantly.

for AA genotype (Table 2). The test day milk yield was significantly ($P \leq 0.05$) highest (11.59 ± 0.51 kg) for AA genotype and lowest (9.77 ± 0.43 kg) for GG genotype (Table 2). The least squares mean of other milk mineral and constituent for the genotypes were non-significantly differing to each other.

Association of rs43691049 with milk mineral and milk constituent traits: The allelic frequency of A and G alleles was 0.200 and 0.800 and the genotypic frequencies of AA, AG and GG genotypes were 0.05 (2 animals), 0.30 (12 animals) and 0.65 (26 animals) respectively. After adjusting the effect of non-genetic factor, Ca was significantly ($P \leq 0.05$) highest (2.01 ± 0.12 mg/l) for AA genotype followed by AG (1.45 ± 0.08 mg/l) and AA (1.46 ± 0.08 mg/l) genotypes (Table 2). However, the associations of genotype were non-significant with other traits under investigation.

Association of rs109727821 with milk mineral and milk constituent traits: The allelic frequency of A and G alleles was 0.650 and 0.350 and the genotypic frequencies of AA, AG and GG genotypes were 0.45 (18 animals), 0.40 (16 animals) and 0.15 (6 animals) respectively. The Mn concentration was significantly ($P \leq 0.001$) highest (1.64 ± 0.16 mg/l) for AG genotype and lowest (0.77 ± 0.24 mg/l) for GG genotype (Table 2). The K content was significantly ($p \leq 0.05$) highest (36 ± 4 meq/l) for GG genotype and lowest (30 ± 1 meq/l) for AA genotype (Table 3). The associations of genotype at rs109727821 locus were non-significant with other milk mineral and constituent traits.

Association of rs109047657 with milk mineral and milk constituent traits: The allelic frequency of A and G alleles was 0.100 and 0.900 and the genotypic frequencies of GG and GA genotypes were 0.80 (32 animals) and 0.20 (8 animals) respectively. The fat percentage was significantly ($P \leq 0.05$) higher ($4.46 \pm 0.14\%$) for GG genotype than AG genotype ($3.89 \pm 0.16\%$) (Table 2). The associations of genotype with other milk mineral and constituent traits were non-significant.

Association of rs135678421 with milk mineral and milk constituent traits: The allelic frequency of G and T alleles was 0.325 and 0.675 and the genotypic frequencies of GG, GT and TT genotypes were 0.075 (3 animals), 0.50

(20 animals) and 0.425 (17 animals) respectively (Supplementary Fig. 2). The associations of genotype at rs135678421 locus were non-significant with any milk mineral and constituent traits.

Similar finding was reported previously, that the effect of rs109421300 was highly significant SNP on fat content in both the Danish Holstein Frisians (DH) and the Danish Jersey (DJ) cattle populations (Buitenhuis *et al.* 2014). Previously, it has been found that *DGAT1* polymorphism was significantly affecting the fatty acid composition and Cu concentration in the bovine milk (Juhlin *et al.* 2012). A polymorphism in exon 8 of the gene encoding acyl CoA:diacylglycerol acyltransferase (*DGAT1*) on *Bos taurus* chromosome 14 (BTA 14), which was associated with increased fat yield, fat and protein percent as well as decreased milk and protein production (Grisart *et al.* 2002). Several other studies have also reported *DGAT* as a significant marker for fat (Cole *et al.* 2011, Raven *et al.* 2013). The effect of rs109421300 was non-significant on other trait (P, Ca, Cu, Mn, Zn, Fe, Na and K) and protein percentage. Similarly, others had also found the effects of the *DGAT1* polymorphism were not significant on yields of Ca, P, and Zn (Grisart *et al.* 2002). The effect of rs109727821 was highly significant ($P < 0.01$) on Mn and non-significant on Ca, Cu, Zn, Fe, Na, K, test day milk yield, fat and protein percentage. In agreement with present finding, rs109727821 was reported to have significant effect on Mn content in milk of Danish HF (Buitenhuis *et al.* 2015). Further, the rs43691049 (BTA 11) was significantly associated with Ca concentration ($P < 0.05$) but it had non-significant association with P, Cu, Mn, Zn, Fe, Na, K, test day milk yield, fat and protein percentage. The rs109047657 located on BTA9, was significantly ($P < 0.05$) affecting fat percentage and non-significantly affecting P, Ca, Cu, Mn, Zn, Fe, Na, K, test day milk yield and protein percentage. Earlier, it was also reported that 11 and 43 SNPs located on BTA 9, were significantly affecting for fatty acid (C6-C10) in DH and DJ cattle, respectively (Buitenhuis *et al.* 2014). The rs135678421 was non-significant ($P < 0.05$) for P, Ca, Cu, Mn, Zn, Fe, Na, K, test day milk yield fat and protein percentage.

Thus it was concluded that in Vrindavani cattle population, the rs109421300 pertaining to Diacylglycerol

Acyltransferase (*DGAT*) gene was significantly affecting the fat percentage and test day milk yield.

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