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Identification of Genetic Variants Influencing Milk Production Traits in Sudanese Dairy Cattle

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Abstract: The aim of study was to investigate the genetic effects of candidate genes for milk production (Diacylglycerol Acyltransferase 1 (DGAT1), Leptin (LEP), kappa casein (CSN3), Growth Hormone Receptor (GHR), Osteopontin (OPN/SPP1) and ATP Binding Cassette subfamily G member 2 (ABCG2)) in Sudanese dairy cattle breeds. We examined forty Butana and twenty Kenana cows for which milk production data were available from two Sudanese research stations. In the DGAT1 K232A, allele 232 K was most frequent with frequencies of 95.31 and 79.17% in Butana and Kenana breeds, respectively. The DGAT1 K232A genotypes showed significant effects on milk yield ($p = 0.0124$), fat content ($p<0.0001$) and protein yield ($p = 0.0361$). The allele substitution effect of the Alanine variant (232A) was significantly higher milk yield (2.45±0.20 kg, $p<0.0001$) and protein yield (0.074±0.01 kg, $p<0.0001$) compared to lysine variant. For the DGAT1 promoter VNTR, three different alleles (3, 4 and 5) were found in Butana cattle and two (3 and 4) in Kenana cattle. Allele 3 was the highest frequent allele of 70.5 and 81.3% in Butana and Kenana breeds, respectively. This allele 3 increased both fat (0.84±0.22%, $p = 0.0013$) and protein (0.19±0.08%, $p = 0.0333$) contents. The same trend was observed in Kenana cattle. For the MboI-RFLP in the *LEP* gene, the allele A was almost fixed with frequencies of 97.50 and 97.06% in Butana and Kenana cows, respectively. The AB carriers had higher milk, protein and fat yields when compared with homozygous AA cows in Butana and Kenana cattle. With respect to CSN3, allele A was the major allele with a frequency of 86.25 and 89.29% for Butana and Kenana cows, respectively. The CSN3 polymorphism was not significantly associated with milk yield and composition. No variation was found for the examined SNPs with *GHR*, *OPN* and *ABCG2* genes. The results of the present study provide evidence that polymorphisms in the *DGAT1*, *Lep* and *CSN3* genes that segregate in Holstein Friesian dairy cattle also segregate in Butana and Kenana cattle and the direction of effect is the same as in Holstein Friesian cattle. These results provide a potential for genetic selection of animals with a predisposition for high milk yield, fat and protein yield in Sudanese cattle. The improve productivity (milk yield) in Sudanese cattle; selection of the minor allele could be enhanced to increased the milk production for these breeds.

Key words: Candidate gene, polymorphism, milk traits, Butana, Kenana, cattle

INTRODUCTION

The improvement of milk yield and composition of dairy cattle is of great interest for African countries, especially where the potential benefits cannot be fully exploited because of suboptimal feeding and housing regimes as for example in tropical and subtropical regions.

In the last decade, studies were conducted to identify genes affecting economically important traits in commercial dairy cattle populations. The major objective of dairy cattle genomics is to identify genes underlying the genetic variability of milk production traits that are useful and could be implemented in breeding programs to meet the growing demands for milk and milk products around the world (Szyda and Komisarek, 2007). Genomic selection offers many advantages with regard to improving the rate of genetic gain in dairy cattle breeding programs and this by increasing the accuracy and intensity of selection and shortening the generation interval, the rate of genetic progress for economically important dairy traits can be approximately doubled (Schefers and Weigel, 2012).

Milk production traits are under the control of many genes, each with a different phenotypic contribution. Because of the complex nature, the identification of the processes and mutations responsible for the large

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genetic variations underlying milk yield and composition proved challenging (Raven *et al*., 2013). The phenotypic information available about milk production traits and the genotypic data for different polymorphisms within diverse candidate genes permit to test the association between the candidate gene variants and milk traits. The resulting information can be used to select the best genotypes and alleles for different traits in future breeding efforts. The incorporation of the molecular genetic information into breeding programs can enhance and improve responses to traditional selection methods (Naqvi, 2007).

Due to the increasing availability of genetic data of exotic dairy cattle breeds, many studies of quantitative traits such as milk production are focusing on hypotheses related to candidate gene approach. However, information about the allelic and genotypic profile at such important functional or positional loci in Sudanese native cattle breeds is hardly available.

The objectives of the present study were to investigate the allelic and distribution pattern of candidate genes (*Acyl-CoA*: Diacylglycerol Acyltransferase 1 (*DGAT1*), Leptin (*Lep/ob*), Kappa Casein (*CSN3*), Growth Hormone Receptor (*GHR*), Osteopontin (*OPN/SPP1*) and ATP Binding Cassette subfamily G member 2 (*ABCG2*)) polymorphisms in two native Sudanese local cattle breeds namely Kenana and Butana dairy cattle. In frame of this study, recognizable efforts were directed not only to carry out different association analyses of the candidate genes with milk yield and composition but also to investigate the genetic variation of candidate genes on milk yield and composition aiming to characterize the genetic polymorphisms.

MATERIALS AND METHODS

Animals: Forty Butana and 20 Kenana cows from Sudan were examined. Cows were kept in two animal production research centers in Sudan, namely the Atbara station (Butana cattle) and the Um-Banein station (Kenana cattle).

Phenotypic data: Milk samples of the Butana and Kenana cows were collected from the Atbara and Um-Banein stations three times per month in the period from the end of April until the end of October in 2008. The cows were milked twice a day and milk samples were taken from both morning and evening milk. The collected milk samples were immediately kept on ice container until they were transferred to the Laboratory of the Dairy Chemistry, Faculty of Animal Production, University of Khartoum for fat and protein content analysis. Fat content was determined using the Gerber Method (Bradley *et al*., 1992). Total nitrogen was determined using the Kjeldahl Method (Bradley *et al*., 1992) and the nitrogen content was converted into equivalent protein content using 6.38 as a conversion factor (Karman *et al*., 1986). The chemical tests for fat and protein content were carried out in duplicate measurements. The amount of daily milk was recorded on the day of sample collection. A total of 610 and 298 lactation records from forty and twenty cows of Butana and Kenana dairy cattle respectively were used in this study.

DNA extraction and genotyping: Blood samples from the jugular vein were collected by sterile tubes containing EDTA anticoagulant and dropped onto FTA papers (Whatman International Ltd., UK). A small piece of FTA paper was punched, washed, dried and used directly for Polymerase Chain Reaction (PCR). The Butana and Kenana cattle were genotyped for the DGAT1 K232A, DGAT1 promoter VNTR, Leptin Mbo1-RFLP, GHR F279Y, CSN3 variant, Osteopontin (C/T) substitution and ABCG2 Y581S polymorphisms (Table 1).

Genotypes were determined using PCR-RFLP for DGAT1 K232A, Lep Mbo1, CSN3 and OPN variants (Table 2). DGAT1 promoter VNTR polymorphism was genotyped by using microsatellite in LI-COR (Licor Biosciences, Nebraska, USA) while GHR F279Y and ABCG2 Y581S were determined by using pyrosequencing in pyrosequencer PSQTM 96MA (Biotage, Uppsala, Sweden) (Table 2). Primers for the PCR were established for GHR F279Y and ABCG2 Y581S from gene sequences available in GenBank database (accession number AM161140.1) and (AJ871176.1), respectively with the use of primer 3 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). The PCR reaction mixture contained a total of 24-96 ng of genomic DNA, 0.4-5 units

Table 1: Description of the polymorphisms analysed in the current study

Genes	DNA polymorphism	Polymorphism ID	Gene region	Location	Position (bp)	Reference for polymorphisms
DGAT1	K232A	rs109234250	Exon 8	BTA14	1802265	Winter *et al.* (2002)
		rs109326954			1802266	
DGAT1	promoter VNTR	_	Promoter	BTA14	1793316	Kuhn *et al.* (2004)
Lep	Mbo1-RFLP	rs109414345	Intron 2	BTA04	93250208	Liefers *et al.* (2002)
GHR	F279Y	rs385640152	Exon 8	BTA20	31909478	Blott *et al.* (2003)
CSN3	A/B variant	rs110870535	Exon 4	BTA06	87390458	Medrano and
		rs43706475			87390479	Aquilar-Cordova (1990)
OPN	C/T variants	rs109072648	Intron 5	BTA06	38122523	Leonard *et al.* (2005)
ABCG2	Y581S	rs43702337	Exon 14	BTA06	38027010	Cohen-Zinder *et al.* (2005)

Table 2: PCR based assay for the detection of the polymorphism

Genes	Polymorphism	Assay	PCR primers (5'-3')	Annealing temp. (°C)	PCR product size (bp)	Restriction enzyme	References
DGAT1	K232A	PCR-RFLP	F: GCACCATCCTCTTCCTCAAG R: GGAAGCGCTTTCGGATG	60	411	CfrI	Winter *et al.* (2002)
DGAT1	Promoter VNTR	Microsatellite	F: TCAGGATCCAGAGGTACCAG R: GGGGTCCAAGGTTGATACAG`	55	228	-	Kuhn *et al.* (2004)
Lep	Mbo1-RFLP	PCR-RFLP	F: TGGAGTGGCTTGTTATTTTCTTCT R: GTCCCCGCTTCTGGCTACCTAACT	55	400	MboI	Liefers *et al.* (2002)
GHR	F279Y	Pyrosequencer	F: TTTGGAATACTTGGGCTAGCA R: AATAACTGGCAAAACATATCAGAGT S: GGCT AGCAGTGACATTAT	57	102	-	This study
CSN3	A/B variant	PCR-RFLP	F: ATCATTTATGGCCATTCCACCAAAG R: GGCCATTTCGCCTTGCTGTAACAGA	56	350	HinfI	Medrano and Aquilar-Cordova (1990)
OPN	C/T variants	PCR-RFLP	F: GCAAATCAGAAGTGTGATAGAC R: CCAAGCCAAACGTTATGAGTT	56	290	Bsr1	Leonard *et al.* (2005)
ABCG2	Y581S	Pyrosequencer	F: ATACTTGAGCATTCCTCGATACG R: AACCAGCACGGTGACAGATA S: CGGTGACAGA TAAGGAGA	57	63	-	This study

of Tag polymerase (Genaxxon BioScience, Germany), 1×PCR buffer, 2-2.5 mM $MgCl_2$, 5 pmol of each primer (Carl Roth GmbH, Karlsruhe, Germany) and 0.1-0.2 mM dNTPs (Carl Roth GmbH Karlsruhe, Germany). The primer sequences, annealing temperature, PCR product size and restriction enzyme are listed in Table 2.

Statistical analyses: For phenotypic data, mean, standard deviation, maximum and minimum was calculated with the PROC MEANS procedure of the Statistical Analysis Software (SAS Institute Inc., 2008, Version 9.1).

The allele and genotype frequencies were estimated based on the counting method (Falconer, 1960). The χ^2-test for Hardy-Weinberg Equilibrium (HWE) was applied to assess the deviation of the number of observed genotypes from the expected genotypes.

In the Butana cow population, we performed association analysis between the genotypes of polymorphism loci in the *DGAT1* and *CSN3* genes and the milk production traits (milk yield, fat yield, protein yield, fat content and protein content). The analysis was performed with a mixed model using the SAS procedure (Model 1) (SAS Institute Inc., 2008, Version 9.1).

The repeatability measures model was used which included the genotype, the combined effect of year and season of calving, lactation and the date of the test day as fixed effects. The four covariates were used in this model to account for the shape of the lactation curve. The lactations were divided into two groups; group one included the first lactation of all cows and group two included the second, third and fourth lactations of all cows. For the estimation of DGAT1 promoter VNTR genotype effects on milk performance trait in Butane cattle, the VNTR genotypes 44 and 45 were excluded from the association analysis because the number of individuals was less than five. The association analyses between the Leptin Mbo1-RFLP genotypes and milk production traits were not performed in Butana and Kenana dairy cattle due to the absence of BB homozygous and low number of AB heterozygous. However, the means of the AA homozygote and AB heterozygote were calculated for Butana and Kenana dairy cattle. The association analysis was not performed for the Kenana cattle because the population was too small. Therefore, only the mean values of the different genotypes were calculated using PROC MEANS (SAS Institute Inc., 2008, Version 9.1).

$$Y_{ijklmn} = \mu + GT_i + YSC_j + L_k + Date_l + X_1 + X_2 + X_3 + X_4 + A_m + \varepsilon_{ijklmn} \quad (1)$$

Where:

$Y_{ijklmnn}$ = Milk production traits (milk yield, fat yield, fat content, protein yield and protein content)
μ = Overall mean of observations
GT_i = Fixed effect of genotypes (i = number of genotypes at gene locus)
YSC_j = Fixed combined effect of year and season of calving (j = 2)
L_k = Fixed effect of lactation (k = 2)
$Date_l$ = Fixed effect of date of the test (l = 19)
X_1 = Days in milk (DIM)/c (as a covariable) where c is a constant set to 305
X_2 = $(DIM/c)^2$ as a covariable
X_3 = log (c/DIM) as a covariable
X_4 = $(\log (c/DIM))^2$ as a covariable
A_m = Random effect of animal
ε_{ijklmn} = Residual error

The allele substitution effects of DGAT1 promoter VNTR and CSN3 polymorphisms on the milk traits were calculated using Eq. 2:

$$Y_{ijklmn} = \mu + (b_i \times x_i) + Y_{SCj} + L_k + Date_l + X_1 + X_2 + X_3 + X_4 + A_m + \varepsilon_{ijklmn} \quad (2)$$

Where:

Y_{ijklmn} = Milk production traits (milk yield, fat yield, fat content, protein yield, protein content)
μ = Overall mean of observations
b_i = Estimated allele substitution effect
x_i = The number of copies at each mutation (0, 1, 2)
YSC_j = Fixed combined effect of year and season of calving (j = 2)
L_k = Fixed effect of lactation (k = 2)
$Date_l$ = Fixed effect of date of the test (1 = 19)
X_1 = Days in Milk (DIM)/c (as a covariable) where c is a constant set to 305
X_2 = $(DIM/c)^2$ as a covariable
X_3 = log (c/DIM) as a covariable
X_4 = $(\log (c/DIM))^2$ as a covariable
A_m = Random effect of animal
ε_{ijklmn} = Residual error

In testing the association and allele substitution effects, Tukey-Kramer correction was applied for multiple testing. The $p \leq 0.05$ was considered significant.

RESULTS

Characterization of Butana and Kenana dairy cattle for milk traits: The average daily milk yields (±SD) for Butana and Kenana cattle over all lactations were 5.81±1.39 and 5.65±0.95 kg, respectively. The corresponding daily fat and protein yields were 0.24±0.08 and 0.21±0.05 kg in Butana cattle, respectively and 0.26±0.06 and 0.20±0.03 kg in Kenana cattle, respectively. The mean (±SD) fat and protein contents were 4.05±0.82 and 3.53±0.31% in Butana cows and 4.68±0.98 and 3.52±0.31% in Kenana cattle, respectively. Although, the Butana and Kenana, cows were kept in different stations under different management and feeding conditions and thus cannot be compared directly in their performance, data shows that both populations produced milk on a similar production level (Table 3).

Table 3: Milk performance in Butana and Kenana dairy cattle per day

Breeds	Traits	Lactation	No. of records	No. of cows	Mean	Min.	Max.	SD
Butana	Milk yield (kg, day)	1	160	11	5.24	2.00	8.70	1.29
		2	44	3	5.63	5.00	6.00	0.32
		3	200	13	6.12	3.50	10.30	1.37
		4	206	13	5.98	3.20	9.20	1.48
	Fat yield (kg, day)	1	160	11	0.21	0.06	0.29	0.05
		2	44	3	0.24	0.16	0.34	0.07
		3	200	13	0.26	0.11	0.57	0.10
		4	206	13	0.23	0.11	0.41	0.07
	Protein yield (kg, day)	1	160	11	0.19	0.07	0.35	0.05
		2	44	3	0.21	0.16	0.24	0.03
		3	200	13	0.22	0.13	0.34	0.05
		4	206	13	0.21	0.11	0.31	0.05
	Fat content (%)	1	160	11	3.98	2.97	5.08	0.66
		2	44	3	4.28	3.00	5.63	1.11
		3	200	13	4.22	3.02	5.83	0.90
		4	206	13	3.90	2.87	5.00	0.73
	Protein content (%)	1	160	11	3.54	3.00	4.30	0.30
		2	44	3	3.72	3.17	4.12	0.37
		3	200	13	3.57	3.10	4.30	0.27
		4	206	13	3.46	2.95	3.90	0.31
Kenana	Milk yield (kg, day)	1	68	4	5.19	3.90	6.40	0.64
		2	134	8	5.69	3.97	7.00	0.72
		3	81	6	5.91	4.12	8.22	1.37
		4	15	2	5.95	5.70	6.20	0.16
	Fat yield (kg, day)	1	68	4	0.23	0.16	0.31	0.05
		2	134	8	0.26	0.17	0.39	0.05
		3	81	6	0.30	0.21	0.45	0.06
		4	15	2	0.21	0.20	0.29	0.01
	Protein yield (kg, day)	1	68	4	0.18	0.15	0.24	0.03
		2	134	8	0.20	0.15	0.25	0.02
		3	81	6	0.21	0.14	0.29	0.05
		4	15	2	0.21	0.20	0.22	0.01
	Fat content (%)	1	68	4	4.41	3.20	4.95	0.75
		2	134	8	4.62	3.00	6.30	1.09
		3	81	6	5.21	3.80	6.05	0.70
		4	15	2	3.52	3.50	3.55	0.02
	Protein content (%)	1	68	4	3.55	2.93	3.91	0.37
		2	134	8	3.47	3.06	3.91	0.32
		3	81	6	3.55	2.19	3.90	0.27
		4	15	2	3.56	3.46	3.62	0.05

No: The number; min.: The minimum value; max.: The maximum value; SD: The Standard Deviation

Table 4: Allele and genotype frequencies of the DGAT1 K232A, DGAT1 promoter VNTR, Leptin Mbo1-RFLP and *CSN3* gene variants polymorphisms in Butana and Kenana dairy cattle

		Genotype frequencies			Allele frequencies	
Locus	Genotype	Butana (n = 40)	Kenana (n = 20)	Allele	Butana	Kenana
DGAT1 K232A	KK	90.63	58.33	232K	95.31	79.17
	KA	9.37	41.67	232A	4.69	20.83
	AA	-	-			
DGAT1 VNTR	33	50.00	62.50	3	70.46	81.25
	34	40.90	37.50	4	27.27	18.75
	44	4.55	-	5	2.27	-
	45	4.55	-			
Leptin Mbo1-RFLP	AA	95.00	94.12	A	97.50	97.06
	AB	5.00	5.88	B	2.50	2.94
	BB	-	-			
CSN3 (A/B variant)	AA	72.50	78.57	A	86.25	89.29
	AB	27.50	21.43	B	13.75	10.71
	BB	-	-			

Genotype and allele frequencies of the candidate gene: Two alleles were found in the DGAT1 K232A polymorphism in Butana and Kenana dairy cattle population and were denoted as 232K for the Lysine variant and 232A for the Alanine variant. The allele 232K was the most frequent allele with the frequency of 95.31 and 79.17% in Butana and Kenana cattle, respectively.

Three different alleles contained 5, 6 and 7 repetitions of the 18 bp repeat motif at the DGAT1 promoter VNTR were segregating in Butana cattle while only two alleles contained 5 and 6 repeat were found in Kenana cattle. The VNTR allele 3 (5 repeats) at the DGAT1 promoter VNTR polymorphism was the highest frequent in both breeds and the frequency ranged between 70.46 and 81.25% in Butana and Kenana cattle, respectively. The VNTR allele 4 occurred with frequencies of 27.27 and 18.75% for Butana and Kenana cattle, respectively (Table 4).

For the Leptin Mbo1-RFLP polymorphism, the digestion of the 400 bp PCR products allowed the discrimination of the two alleles A and B. The frequencies of allele A were 97.50 and 97.06% in Butana and Kenana breeds, respectively.

In the kappa casein locus, the digestion of the 350 bp PCR product with the restriction enzyme (Hinf1) revealed the alleles A and B. The frequencies of allele A for the two breeds were 86.25 and 89.29% in Butana and Kenana cows, respectively (Table 4).

The examined Butana and Kenana cows population were fixed for the 279F allele of the *GHR* gene for T allele in the *OPN* gene and for A allele in the *ABCG2* gene.

The observed genotypes in the examined cow populations were in Hardy-Weinberg-equilibrium for *DGAT1*, *Lep* and *CSN3* genes in Butana and Kenana cattle.

Effects of candidate genes genotypes on milk production traits: The DGAT1 K232A genotypes showed significant effects on milk yield ($p = 0.0124$), protein yield ($p = 0.0361$) and fat content ($p<0.0001$) in Butana cattle. The results of the difference between DGAT1 K232A genotype effects are presented in Table 5. The KK homozygous genotype of lysine had pronounced effects especially in fat content. The KA heterozygous cows had significantly higher milk and protein yield than the KK homozygous cows. For the fat yield and protein content, there were no significant differences between KK homozygous and KA heterozygous cows.

The effect of DGAT1 promoter VNTR polymorphism on milk production traits in Butana dairy cattle breed revealed that it had significant ($p = 0.0103$) effects on fat content. The effect was not significant for milk yield, fat yield as well as protein yield and content in Butana cows. The homozygous genotype 33 was significantly associated with high fat content when compared with the heterozygous genotype 34. Moreover, cows with the 33 genotypes produced 23 and 4% fat yield and protein content, respectively than cows with 34 genotypes. These differences were however not significant. The genotype 34 had higher milk and protein yields than the 33 homozygous cows (Table 5). The number of the samples which represented the different genotypes of the DGAT1 K232A and DGAT1 promoter VNTR in Kenana dairy breed was too small. The means of the KK and KA genotypes at DGAT1 K232A and 33 and 34 at DGAT1 promoter VNTR on milk yield and composition were documented. For the genotype differences similar trend was observed in Kenana dairy cattle.

The association analyses between the Leptin Mbo1-RFLP genotypes and milk production traits showed that AB heterozygous cows were higher for milk, protein and fat yields when compared to AA homozygous cows in both analyzed breeds.

No significant associations were recognized between CSN3 genotypes and all analyzed milk production traits.

Table 5: Differences between genotypes at the DGAT1 K232A, DGAT1 promoter VNTR and CSN3 loci (LSM, SE, p-values) in Butana dairy cattle

		Genotype differences (KK-KA)		
Locus	Milk traits	Estimate	SE	p-values
DGAT1 K232A	Milk yield (kg)	2.42	0.88	0.0124
	Fat yield (kg)	0.02	0.05	0.7351
	Protein yield (kg)	0.08	0.04	0.0361
	Fat content (%)	-1.07	0.11	<0.0001
	Protein content (%)	-0.10	0.22	0.6449
	Milk traits	Genotype differences (33-34)		
DGAT1 VNTR	Milk yield (kg)	-0.38	0.70	0.5960
	Fat yield (kg)	0.05	0.03	0.2092
	Protein yield (kg)	-0.01	0.03	0.8562
	Fat content (%)	0.93	0.32	0.0103
	Protein content (%)	0.16	0.12	0.2034
	Milk traits	Genotype differences (AA-AB)		
CSN3 (A/B variant)	Milk yield (kg)	-0.37	0.49	0.4480
	Fat yield (kg)	-0.04	0.03	0.1563
	Protein yield (kg)	-0.02	0.02	0.1920
	Fat content (%)	-0.59	0.29	0.0502
	Protein content (%)	-0.19	0.11	0.0935

Adjusted p-value in accordance with Tukey-Kramer ($p<0.05$)

Table 6: Least Square Means (LSM) and Standard Error (±SE) of DGAT1 K232A, DGAT1 VNTR and CSN3 (A/B variant) genotypes and means of Leptin Mbo1-RFLP on milk production traits in Butana cattle

		Milk traits				
Locus	Genotype	MY	FY	PY	FC	PC
DGAT1 K232A	KK	5.46±0.31	0.23±0.02	0.20±0.01	4.13±0.08	3.62±0.08
	KA	7.88±0.87	0.25±0.05	0.28±0.04	3.07±0.13	3.51±0.22
DGAT1 VNTR	33	5.54±0.61	0.27±0.03	0.21±0.02	4.56±0.28	3.81±0.11
	34	5.92±0.58	0.22±0.03	0.22±0.02	3.63±0.27	3.65±0.10
Leptin Mbo1-RFLP	AA	5.69±0.05	0.24±0.00	0.20±0.00	4.09±0.03	3.53±0.01
	AB	8.42±0.07	0.28±0.01	0.31±0.00	3.31±0.04	3.66±0.06
CSN3 (A/B variant)	AA	5.50±0.32	0.22±0.02	0.19±0.01	3.96±0.18	3.52±0.07
	AB	5.87±0.42	0.26±0.02	0.22±0.02	4.55±0.25	3.71±0.10

Table 7: Means and Standard Error (±SE) of DGAT1 K232A, DGAT1 VNTR, Leptin Mbo1-RFLP and CSN3 (A/B variant) genotypes on milk production traits in Kenana cattle

		Milk traits				
Locus	Genotype	MY	FY	PY	FC	PC
DGAT1 K232A	KK	5.41±0.08	0.27±0.01	0.20±0.00	5.09±0.09	3.71±0.02
	KA	5.95±0.12	0.28±0.01	0.21±0.00	4.67±0.11	3.54±0.03
DGAT1 VNTR	33	5.32±0.13	0.28±0.01	0.20±0.04	5.29±0.06	3.78±0.01
	34	6.05±0.07	0.27±0.01	0.21±0.00	4.84±0.12	3.55±0.04
Leptin Mbo1-RFLP	AA	5.50±0.06	0.25±0.00	0.19±0.00	4.63±0.07	3.53±0.02
	AB	7.53±0.11	0.40±0.01	0.27±0.00	5.34±0.07	3.58±0.03
CSN3 (A/B variant)	AA	6.09±0.07	0.27±0.00	0.21±0.00	4.50±0.07	3.50±0.02
	AB	5.26±0.10	0.30±0.01	0.20±0.00	5.57±0.08	3.82±0.02

MY: Milk Yield/kg, FY: Fat Yield/kg, PY: Protein Yield/kg, FC: Fat Content/% and PC: Protein Content/%

The differences between genotype classes of CSN3 genotypes in Butana dairy cows are presented in Table 5. The milk from cows with the AB heterozygous genotypes had marginally higher fat content (0.59±0.29%) and higher protein content (0.19±0.11%) than the AA genotype cows. The AB heterozygous genotypes produced more yield of milk, fat and protein compared to the AA homozygous cows although not significant. No association analyses were performed on Kenana dairy cattle. Only the means were obtained for the different genotype groups. The means of the AB heterozygous genotypes were higher for fat and protein contents compared to the AA homozygous group in Kenana cattle.

The least square means for DGAT1 K232A, DGAT1 promoter VNTR, CSN3 and means of Lep Mbo1 RFLP genotypes for milk traits in Butana cows are presented in Table 6 and 7.

Allele substitution effects: The estimated allele substitution effects for the alanine variant at DGAT1

Table 8: Allele substitution effects (α) of the 232A at DGAT1, VNTR allele 3 at DGAT1 and allele B at CSN3 loci, Standard Errors (SE) and p-value for milk traits in Butana dairy cows

		232A		
Locus	Milk traits	α	SE	p-values
DGAT1 K232A	Milk yield (kg)	2.45	0.20	<0.0001
	Fat yield (kg)	0.02	0.01	0.0708
	Protein yield (kg)	0.07	0.01	<0.0001
	Fat content (%)	-0.07	0.11	<0.0001
	Protein content (%)	-0.20	0.05	<0.0001
	Milk traits	VNTR-Allele 3		
DGAT1 VNTR	Milk yield (kg)	-0.24	0.49	0.6329
	Fat yield (kg)	0.04	0.02	0.0923
	Protein yield (kg)	0.002	0.02	0.9331
	Fat content (%)	0.84	0.22	0.0013
	Protein content (%)	0.19	0.08	0.0333
	Milk traits	Allele B		
CSN3 (A/B variant)	Milk yield (kg)	0.37	0.49	0.4480
	Fat yield (kg)	0.04	0.03	0.1563
	Protein yield (kg)	0.02	0.02	0.1920
	Fat content (%)	0.59	0.29	0.0502
	Protein content (%)	0.20	0.11	0.0935

K232A were 2.45±0.20 and 0.07±0.01 kg for milk and protein yield, respectively. Negative effects of the alanine variant were found for the fat and protein content (Table 8).

The VNTR allele 3 at *DGAT1* gene contributed significantly to increased fat content (0.84±0.22%) and protein content (0.19±0.08%) in Butana dairy cows.

The allele substitution effects of the variant B in the *CSN3* gene showed no significant values. However, all the traits were positively influenced by the B allele of the *CSN3* gene with the positive increase in fat and protein contents in Butana cattle by 0.59±0.29 and 0.20±0.11%, respectively.

DISCUSSION

In developing countries, the performance of the imported high-yielding breeds such as Holstein Friesian which is the most widely used exotic breed in the tropics is often negatively affected by genotype-environment interactions (Elseed *et al*., 2008; Ojango *et al*., 2005; Rege, 1991). Therefore, indigenous breeds like Butana and Kenana dairy cattle are promising for milk production. These breeds produced under controlled feeding and management conditions in research stations about 1500 kg milk per lactation (EL-Habeeb, 1991; Musa *et al*., 2006; Lutfi *et al*., 2005).

The genetic improvement of these breeds for high milk production could increase their local values of the breed and improved the performance levels. However, effective genetic improvement requires genetic information about the genetic variability and their effects on milk production. To estimate genetic effects, performance testing and genotypic data are necessary.

To perform an association analysis in tropical countries like Sudan is not easy because the basic prerequisites for those analyses like milk recording do still not exist and their use in organized breeding program (Syrstad and Ruane, 1998; Thornton, 2010). Therefore, the current study is the first attempt to do such association analysis in Sudanese dairy cattle even if the number of animals which were phenotype and genotyped is low.

In this study, two loci of the *DGAT1* gene, the amino acid substitution (K232A) and the promoter VNTR were analyzed. The DGAT1 K232A had strong effects on milk yield, protein yield and fat content in Butana dairy cattle. These results provide confirmation of previous findings in other dairy cattle populations (Bennewitz *et al*., 2004; Berry *et al*., 2010; Grisart *et al*., 2002; Kuehn *et al*., 2007; Kuhn *et al*., 2004; Rahmatalla *et al*., 2008; Sanders *et al*., 2006; Thaller *et al*., 2003; Winter *et al*., 2002). The lysine homozygous cows produced significantly more fat content than heterozygous cows while the KA heterozygous cows produced significantly more milk and protein yield. This large magnitude of the effect of the K232A amino acid substitution which was found in a similar magnitude and direction of effect in different studies supports the assumption that this mutation is a causal mutation or Quantitative Trait Nucleotide (QTN) (Mackay, 2001). The current study shows that the Alanine variant increased milk and protein yields while the lysine variant had a contrary effect. These results were consistent with respect to their direction but the magnitude of the estimated effects changed from one population to another and from one breed to another.

The combination of allele substitution effects and allele frequency gives an indication on the genetic variance due to the DGAT1 K232A polymorphism. The estimated allele frequency at DGAT1 K232A was 95.3 and 79.17% for the lysine variants in the Butana and Kenana cattle population, respectively. Mashhadi *et al*. (2012) reported an allele frequencies of 79.6% of the lysine variant and 20.3% for the alanine variant in Iranian Holstein bull (Tantia *et al*., 2006).

The DGAT1 promoter VNTR in the 5' non-coding region of DGAT1 has been proposed to explain the additional part of the QTL variance and one allele (allele 5) was suggested as a putative causal allele for fat yield and content in the dairy cattle (Kuhn *et al*., 2004; Sanders *et al*., 2006). In this study, this allele was only observed in one cow in Butana cattle and was absent in Kenana cattle. Therefore, its effects were not estimable in the two breeds. Nevertheless, the DGAT1 promoter VNTR genotypes significantly affected the content of fat in Butana dairy cattle ($p = 0.0103$). The VNTR allele 3 significantly increased the fat content by 0.84±0.22% and the content of protein by 0.19±0.08% and it had a tendency to increase the yield of fat by 0.04±0.02 kg. The effects of VNTR allele 3 were consistent with the differences between the genotypes for fat and protein contents as well as for fat yield. The homozygous genotypes 33 showed a higher content of fat and protein and fat yield than the heterozygous genotypes 34 (Table 4) in Butana breed. The same trend was also observed in Kenana cattle (Table 5). It means that the VNTR allele 3 at the DGAT1 promoter VNTR was superior regarding its influence on the contents of fat and protein and yield of fat in Butana cattle. As mentioned above, the functional analysis by Furbass *et al*. (2006) found that the promoter VNTR polymorphism affects the number of potential sp1 binding sites and therefore might have an impact on DGAT1 expression and also on the content of fat in milk. Nevertheless, non-significant differences in the stimulating effects of three different alleles (allele 3-5) were observed in the study by Furbass *et al*. (2006). The result for the VNTR allele 3 in Butana cattle was found to be the most likely and is in agreement with our pervious study in German Holstein Friesian cows (Rahmatalla *et al*., 2008). The VNTR-allele 3 in Holstein cows significantly increased the fat yield and it showed tendency to an increased the fat content in milk (Rahmatalla *et al*., 2008). Furthermore, Gautier *et al*. (2007) concluded the effect of the different alleles of the VNTR polymorphism which was associated with milk yield, fat and protein yield and fat and protein content in Montbe'liarde, Normande and French Hostein breed.

For the intronic mutation in the *Leptin* gene, almost similar allele and genotype frequencies were observed in Butana and Kenana dairy cattle but the frequency of allele B was very low in both breeds (Table 3). It was found that only 2 out of 40 of Butana cattle and one out of 17 of Kenana cattle carried the AB heterozygous genotype. BB homozygotes were absent from the two breeds. In Sahiwal cattle (best dairy breeds in India and Pakistan), Dandapat *et al*. (2010) found only allele A at the Leptin locus. In Iranian Holstein cattle, Moussavi *et al*. (2006) found that AA and AB genotypes had, respective frequencies of 0.86 and 0.14 while BB genotypes were not observed. The absence of BB genotypes in Butana and Kenana cattle in this study may be attributed to the small size of the sample for each breed. The mean of AB heterozygous cow in Butana and Kenana cattle was higher on milk, fat and protein yields than the AA homozygous. Therefore, a further association analysis regarding this polymorphism in Butana and Kenana dairy cattle should be considered.

In Kappa casein gene, the frequency of the B allele in Butana and Kenana cattle was estimated as 13.75 and 10.71%, respectively. There was no homozygous BB carrier in Butana and Kenana cattle. The estimated allele frequency of allele B was lower than in Sahiwal (*Bos indicus*) cattle which was 16% in the study by Mitra *et al*. (1998). The frequency of CSN3 allele A in this study was found to be higher than that of allele B which is in close agreement to the results of earlier studies performed in Bos taurus (Kemenes *et al*., 1999; Ng-Kwai-Hang *et al*., 1984; Pinder *et al*., 1991). The frequencies of AA and AB genotypes in Butana and Kenana cattle breed were similar to the frequencies of 75.8, 23.0% for Sahiwal and 73.2 and 25.0% for Tharparkar, respectively (Rachagani and Gupta, 2008). In Egyptian Baladi cattle, Gouda *et al*. (2013) reported the detection of AA and AB genotypes with absence of BB homozygote with a higher frequency of A allele (0.63%) in the CSN3 variation. The AB heterozygous cows at CSN3 locus produced a higher fat and protein content in Butana cattle than the AA homozygous cows (Table 4). This positive effect is attributed to the effect of allele B on the fat and protein contents increase by 0.59±0.29 and 0.20±0.11%, respectively. A positive trend for AB heterozygous animals on fat and protein contents was also found in Kenana dairy cattle. These results are similar to those reported by Botaro *et al*. (2009) who found that milk fat was influenced by CSN3 variants and was higher in milk of AB heterozygotes in Holstein and Girolando cows. Ng-Kwai-Hang (1997) found higher fat and protein contents in cows carrying the CSN3 B allele. The no significant association between CSN3 genotypes in this study was consistent with these reported by Trakovicka *et al*. (2012) who showed the results from the statistical analysis between CSN3 genotypes and milk yield and protein and fat yield in Simmental and Holstein cows were not significant.

The amino acid polymorphism F279Y in the transmembrane domain of the GHR has been suggested as the putative cause for the lactation related QTL effect or to be at least a tightly linked polymorphism (Blott *et al*., 2003). The Tyrosine allele (279Y) was absent in Butana

and Kenana dairy cattle which is in agreement with the result by Varvio *et al.* (2008) who noticed the absence of the 279Y allele in East African, Danish Red and Northern Finn cattle breeds. In our previous study in German Holstein dairy cows, we mention that the 279Y was the minor allele with a frequency of 16.5% (Rahmatalla *et al.*, 2011) and this is an agreement with other Holstein populations (Blott *et al.*, 2003; Oikonomou *et al.*, 2009).

In the C/T substitution of the *OPN* gene, all the genotyped cows were TT homozygous in Butana and Kenana dairy cattle. The CC genotype of the *OPN* gene was low in red cattle in the South and East Anatolian (Oztabak *et al.*, 2008) and Czech Fleckvieh cows (Boleckova *et al.*, 2012).

The genotype testing of the *ABCG2* gene in Butana and Kenana cattle indicated that this polymorphism was not segregating in the two populations. All genotyped cattle had the AA genotype. This result indicated that this polymorphism had no apparent use for marker assisted selection in Butana and Kenana cattle. The analysis of ABCG2 polymorphism in Indian breeds of cattle (*Bos indicus*) revealed also a fixed ABCG2 allele A (Tantia *et al.*, 2006). In another study, the allele frequencies of ABCG2 polymorphism were determined in 32 *Bos taurus* and 3 *Bos indicus* breeds. The ABCG2 allele A was predominant in all populations and it approached the fixation in 23 out of the 35 breeds including all 3 *Bos indicus* breeds (Ron *et al.*, 2006).

CONCLUSION

The overall outcome of the current study revealed that the Sudanese dairy cattle (Butana cattle) showed an association between genetic variants at the DGAT1 K232A and milk and protein yield and fat content and with the promoter region of *DGAT1* gene and fat content. The Alanine variant which is the minor allele at DGAT1 K232A could attribute to increase the milk and protein productivity in Sudanese dairy cattle. Furthermore, allele 3 of the VNTR in the promoter of the *DGAT1* gene appeared favourable for fat and protein contents of milk and fat yield in the investigated Sudanese dairy cattle. However, to draw final conclusions, it is recommended to extend these studies to a larger population.

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