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Variants of the *Diacylglycerol acyltransferase 1* (*Dgat1*) Gene in Sudanese Dairy Cattle (Kenana and Butana)

[1]S.M. Abu Sara, [3]A. Said Ahmed, [2]M.K.A. Ahmed, [3]M. Reissmann,
[3]G.A. Brockmann and [2]S.A. Rahmatalla
[1]Department of Animal Production,
Genetic and Animal Breeding, Neelain University, Khartoum, Sudan
[2]Departments of Dairy Production and Animal Breeding, University of Khartoum, Khartoum, Sudan
[3]Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences,
Humboldt-Universitat zu Berlin, Berlin, Germany

Abstract: The aim of the study was the characterization of DGAT1 variants in Sudanese dairy cattle breeds. In this study, we examined 94 Kenana and 91 Butana dairy cattle from two regions of Sudan. We genotyped the DGAT1 sequence variant AJ318490.1:g.10433/10434 AA>GC that leads to the Lysine-Alanine substitution at position 232 (K232A) in the protein and the VNTR polymorphism in the promoter region. Genotyping was performed by allele specific PCR and PCR fragment lengths determination, respectively. In both breeds, the DGAT1 Lysine variant (232K) that is associated with high fat and protein content as well as high fat yield in other breeds is the high frequent allele. The frequencies of the 232K allele were 96.3 and 84.6% in Kenana and Butana breeds, respectively. At the DGAT1 promoter VNTR locus, four alleles containing four to seven repeats of the 18 bp motif were found in both breeds. The highest frequent allele was the VNTR allele 3 containing five repeats with 60.4 and 57.5% in Kenana and Butana breeds, respectively. In conclusion, the two examined Sudanese dairy cattle breeds do not differ in allele frequencies at the DGAT1 locus.

Key words: Dairy cattle, DGAT1, Kenana, Butana

INTRODUCTION

The cattle population in Sudan was estimated to be 29, 210, 47 head. The increasing demand for fresh milk and milk products requires the improvement of the productivity of dairy breeds. Among them, indigenous breeds that are adapted to the local environmental conditions are of particular value for milk production. Kenana and Butana are such indigenous dairy breeds that belong to the large East African *Bos indicus* breeds. Kenana cattle are distributed East of the confluence of the Blue and White Niles, down the Eastern bank of the Blue Nile up to the Ethiopian border and down the Western bank in the Gezira region South of Khartoum. The Butana breed is native to the Butana region East of Khartoum which extends to the desert area between the Blue Nile and the Atbara River.

Under high feeding and management condition of research stations in Sudan, Kenana and Butana cattle can produce >1500 kg milk per lactation (EL-Habeeb, 1991; Lutfi *et al*., 2005; Saeed *et al*., 1987). Among several candidate genes, the Diacylglycerol Acyltransferase1 (DGAT1) became a functional candidate gene for lactation traits after studies indicated that female knockout mice lacking DGAT1 did not lactate due to the interrupted triglyceride metabolism in the mammary gland (Smith *et al*., 2000).

The *DGAT1* gene was mapped on bovine chromosome 14 close to the centromere. It spans 14,117 bp and comprises 17 exons (Winter *et al*., 2002). The non-conservative substitution of Lysine by Alanine K232A in the *DGAT1* gene which is caused by a sequence variation of the two bases Adenine/Adenine to Guanine/Cytosine at positions 10433 and 10434 in exon 8 (rs109234250, rs109326954) had strong effects on milk yield and composition in several breeds and different Holstein cattle populations in New Zealand (Farnir *et al*., 2002; Grisart *et al*., 2002), the Netherlands (Farnir *et al*., 2002), Germany (Sanders *et al*., 2006; Thaller *et al*., 2003), Poland (Pareek *et al*., 2005; Strzalkowska *et al*., 2005), France (Gautier *et al*., 2007), Sweden (Naslund *et al*., 2008) and Brazil ((Lacorte *et al*., 2006). Cows homozygous for the Alanine variant had higher milk, protein and lactose yields than the other genotypes. Carriers of the Lysine variant had higher fat yield and higher contents of fat and protein (Thaller *et al*., 2003).

Corresponding Author: Siham A. Rahmatalla, Departments of Dairy Production, University of Khartoum, Shambat P.O. Box 32, Postal Code 13314, Khartoum North, Sudan

Besides the protein variants, a Variable Number of Tandem Repeat (VNTR) motive in the promoter region of the *DGAT1* gene was identified as an additional source of variation for milk yield and composition, especially in milk fat content (Bennewitz *et al*., 2004; Kuhn *et al*., 2004). The VNTR polymorphism contains a SP1 transcription factor binding site motif (CCCGCC) and therefore could have functional relevance for the regulation of gene expression (Kuhn *et al*., 2004). The potential functional relevance of the DGAT1 promoter VNTR alleles is underlined by *in vitro* studies providing evidence for SP1 binding to the CCCGCC motif of the repeat and for differential gene expression activity by different VNTR alleles (Furbass *et al*., 2006). Furthermore, the DGAT1 promoter VNTR allele 5 showed a significantly superior effect on milk fat and milk protein content in Holstein dairy cattle while milk yield was decreased compared to all other promoter VNTR alleles (Kuehn *et al*., 2007). The most frequent allele of the DGAT1 promoter was the VNTR allele 3 (5 repeats) (Kuehn *et al*., 2007; Kuhn *et al*., 2004). This allele showed significant positive effects on fat yield in German Holstein cows.

The aim of this study was to characterize the *DGAT1* gene in the two Sudanese dairy cattle breeds Kenana and Butana in order to obtain information on allele frequencies of DGAT1 polymorphisms for selection decisions to improve the genetic potential in milk production.

MATERIALS AND METHODS

Animals: In this study, 94 Kenana and 91 Butana cattle were used. Blood samples were collected from unrelated individuals according to the recommendations by FAO (2000). Kenana cattle were choosen from Sennar state and Butana cattle from Nile river state. For Kenana cattle, in eight villages 10 samples were collected and 14 samples were collected in one additional village. For Butana breed, 11 samples were collected from each of seven villages and 14 samples were collected in one village.

Genotyping: DNA from blood samples was extracted with the Bioscience kit (Bioscience GmbH, Jena, Germany). The genotyping of the DGAT1 K232A substitution (AJ318490.1:g.10433/10434 AA>GC) in exon 8 was carried out by a competitive allele specific PCR (KASP assay) that has been described in detail previously (Kreuzer *et al*., 2013). Primers for PCR were designed from the *DGAT1* gene sequence available at GenBank (accession number AJ318490.1) using KBioscience software (www.kbioscience.co.uk). The following allele specific primers were used: 5'-GAAGGTGACCAAGTTCATGCTCGTAGCTTTGGCAGGTAAGA-3' (Primer A1) and 5'-GAAGGTCGGAGTCAACGATTCTCGTAGCTTTGGCAGGTAAGG-3' (Primer A2). The reverse primer sequence was 5'-GCTGGGCAGCTCCCCCGTT-3'. PCR was performed in a volume of 8.1 μL containing 30 ng dried genomic DNA, 4.0 μL 2X KASP reaction mix (LGC, Herts, UK), 0.11 μL primer mix (100 μM A1-primer: 100 μM A2-primer:100 μM C-primer:water = 1: 1: 2.5: 4), 0.06 μL 50 mM $MgCl_2$ and 4.0 μL water. The DGAT1 promoter VNTR was genotyped as described by Kuhn *et al*. (2004). The primers left and right of the VNTR were 5'-CAGACGTTGTAAAACGACGACCCTGGCAGCACCTCAATC-3' and 5'-AGAAGGCACGGACTGTGAAGGC-3', respectively. The PCR reaction contained 30 ng genomic DNA in a reaction volume of 15 μL with 0.2 μM of each primer, 2.5 mM $MgCl_2$, 1.0 μL of 10X B buffer, 0.1 mM dNTP, 1.5 μL of solution S 10X and 0.5 U Hot-FirePol taq polymerase (Solis Bio Dyne, Tartu, Estonia). We used the M13 tail technique for fluorescence labelling of the fragments during PCR. After denaturation of PCR-products, the samples were loaded on a 6% polyacrylamide gel and run on a LICOR sequencer (Licor Biosciences, Nebraska, USA). The VNTR comprises an 18 bp repetitive sequence motif. Four VNTR alleles were found which were denoted according to the fragment length with the longest fragment having the lowest number of repeats. The VNTR allele 2 contains four repeats, VNTR allele 3 five, VNTR allele 4 six and VNTR allele 5 seven repeats.

Statistical analysis: Allele and genotype frequencies were calculated based on the counting method (Falconer and Mackay, 1996). The χ^2-test was used to test differences of genotype frequencies between the breeds using MedCalc Software (Schoonjans *et al*., 1995). The χ^2-test was also used for testing Hardy-Weinberg equilibrium.

RESULTS

In Sudanese dairy cattle most of the animals were homozygous for the DGAT1 Lysine variant KK (Table 1). In the examined Kenana and Butana animals, the frequencies of the 232K allele were 96.3 and 84.6%, respectively. Frequencies of the different genotypes are presented in Table 1. With respect to the DGAT1 protein variants, the χ^2-test showed that the examined population of Kenana cattle was in Hardy-Weinberg equilibrium ($\chi^2 = 0.14$) while the population of Butana cattle was not ($\chi^2 = 9.59$). The differences in genotype frequencies between Kenana and Butana cows were marginal ($p = 0.057$).

Table 1: Genotype and allele frequencies of the DGAT1 K232A polymorphism

Breeds	No. of animals	Genotype	Genotype frequency (%)	Allele	Allele frequency (%)	HWE (χ^2-values)
Kenana	94	KK	92.5	232K	96.3	0.14^{NS}
		KA	7.5	-	-	-
		AA	-	232A	3.7	-
Butana	91	KK	75.8	232K	84.6	9.59^{S}
		KA	17.6	-	-	
		AA	6.6	232A	15.4	-

^{NS}No significant deviation from HWE, ^{S}Significant deviation from HWE

Table 2: Genotype and allele frequencies at the DGAT1 VNTR locus

Breeds	No. of animals	Genotype	Genotype frequency (%)	Allele	Allele frequency (%)	HWE (χ^2-values)
Kenana	1	22	1.3	2	3.9	-
	4	23	5.2	3	60.4	-
	39	33	50.6	4	35.1	43.5^{S}
	10	34	13.0	5	0.6	-
	1	35	1.3	-	-	-
	22	44	28.6	-	-	-
Butana	3	23	3.2	2	2.1	-
	1	25	1.1	3	57.5	-
	31	33	33.0	4	39.9	-
	43	34	45.7	5	0.5	45.3^{S}
	16	44	17.0	-	-	-

^{S}Significant deviation from HWE

The DGAT1 promoter VNTR has been proposed to explain additional variance of milk yield and composition. Four different alleles (4-7 repeats of the 18 bp motif) at the DGAT1 promoter VNTR were segregating in Kenana and Butana cows. The most frequent allele in both breeds was the VNTR allele 3 containing five repeat elements. Frequencies of VNTR allele 3 were 60.4 and 57.5% in Kenana and Butana breeds, respectively. The VNTR allele 4 (6 repeats) was present with frequencies of 35.1 and 39.9% in Kenana and Butana cattle, respectively. The promoter VNTR allele 2 with four repeats and allele 5 with seven repeats were least frequent with 3.9 and 0.6%, respectively, in Kenana cows and 2.1 and 0.5%, respectively, in Butana cows (Table 2). For the DGAT1 promoter VNTR polymorphism, significant deviations from Hardy-Weinberg-equilibrium in Kenana and Butana cattle populations were observed. There are significant differences between genotypes in Kenana ana Butana at DGAT1 promoter VNTR ($p<0.0001$).

DISCUSSION

In this study, the estimated allele frequency at DGAT1 K232A was 96.3 and 84.6% for the Lysine and 3.7 and 15.4% for the Alanine variants in Kenana and Butana cattle, respectively. The main zebu breed in Brazil, Gyr and Red Sindhi, showed high frequencies of >96% of the 232K allele, respectively (Lacorte *et al*., 2006). The 232K allele is fixed in Sahiwal, Rathi, Deoni, Tharparkar, Red Kandhari and Punganur Indian *Bos indicus* breeds (Tantia *et al*., 2006), Indian Nellore cattle (Kaupe *et al*., 2004), Brazilian Nellore and Guzerat cattle (Lacorte *et al*., 2006). In the Holstein Friesian breed, frequencies of DGAT1 alleles differed considerably between populations. Thaller *et al*. (2003) reported an allele frequency of 55 and 44.2% of the Lysine variant in German Holstein sires and cows, respectively. For Dutch Holstein Friesian cows and Polish black and white Friesian cows, the allele frequency of 40% for the Lysine variant was estimated by Schennink *et al*. (2008) and Strzalkowska *et al*. (2005). Other studies estimated the allele frequencies between 30 and 70% in the Holstein population and in the Polish Black and White populations (Bovenhuis and Schrooten, 2002; Grisart *et al*., 2002; Pareek *et al*., 2005; Winter *et al*., 2002). The frequency of the Lysine variant was lower (12%) in Swedish Holstein cows (Naslund *et al*., 2008).

In different studied populations for several dairy cattle breeds including Holstein Friesian (Bennewitz *et al*., 2004; Grisart *et al*., 2002; Kuehn *et al*., 2007; Spelman *et al*., 2002; Thaller *et al*., 2003), Jersey (Komisarek *et al*., 2004; Spelman *et al*., 2002), Ayrshire (Spelman *et al*., 2002) and Angeln dairy cattle (Sanders *et al*., 2006), the Lysine variant was consistently associated with high fat and protein contents as well as high fat yield. Although, the magnitude of the effects differed among the populations, the direction of effects was always the same.

With respect to the DGAT1 promoter VNTR alleles, the VNTR allele 3 (five repeats) had the highest frequency of 60.4 and 57.5% in Kenana and Butana cows, respectively. In German Holstein Friesian cows, the promoter VNTR allele 3 (5 repeats) was the most frequent allele with 55% (Kuehn *et al*., 2007) and 62.7% (Rahmatalla *et al*., 2008). The highest frequent allele (VNTR allele 3) found in German Holstein which

accounted for increased fat yield were also found in high frequencies in Kenana and Butana cattle. We would expect that this allele is also associated with the same direction of allele effects as in German Holstein Friesian. However, these expectations must be confirmed in Kenana and Butana cattle. Therefore, it is necessary to record milk performance and composition from animals of the examined populations.

CONCLUSION

From the results obtained, it can be concluded that the Lysine variant of DGAT1 which is associated with high fat and protein content in Holstein cattle was the most frequent allele in both Kenana and Butana cattle. The frequency of VNTR allele 3 (five repeats) of the DGAT1 promoter VNTR polymorphism was high in the examined Sudanese breeds. This allele is also associated with high fat yield in Holstein cattle. The obtained genetic information can be used for studying the effect of allelic association with milk yield and composition traits in Kenana and Butana cattle which is necessary before selection decisions of the minor allele can be drawn for improving the local breeds. Albeit milk production traits in Sudan are not recorded, the DGAT1 genotyping data generated in this study suggest that the low milk yield with the high fat and protein content in Sudanese *Bos indicus* Kenana and Butana cattle compared to taurine cattle could results in part from the genetic predisposition associated with the *DGAT1* gene variants.

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