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Characteristic of the Diacylglycerol Acyltransferase 1 (DGAT1) *K232A* Gene in Sudanese Dairy Cattle (Kenana and Butana)

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Abstract

The objective of the study was the characterization of DGAT1 variant 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. Genotyping was performed by allele specific PCR (KASP assay methods). In both breeds, the DGAT1 Lysine variant (*232KA*) 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. In conclusion, the two examined Sudanese dairy cattle breeds do not differ in allele frequencies at the DGAT1 loci.

Keywords: 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 more than 1500 kg milk per lactation [1]. 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 [2].

The DGAT1 gene was mapped on bovine chromosome 14 close to the centromere. It spans 14,117 bp and comprises 17 exons [3]. 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 [4,5], the Netherlands, Germany [6,7], Poland [8,9], France [10], Sweden and Brazil [11]. 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.

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 of FAO, 1996. Kenana cattle were chosen 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 [12]. 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 MgCl2, and 4.0 µl water.

Statistical analysis

Allele and genotype frequencies were calculated based on the counting

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method. The Chi square test was used to test differences of genotype frequencies between the breeds using MedCalc software [13]. The Chi-square 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* as shown in Table 1. In the examined Kenana and Butana animals, the frequencies of the *232KA* gene 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 Chi-square test showed that the examined population of Kenana cattle was in Hardy-Weinberg equilibrium (χ^2=0.14), while the population of Butana cattle was not (χ^2=9.59). The differences in genotype frequencies between Kenana and Butana cows were marginal (p=0.057) (Table 1).

Table 1. Genotype and allele frequencies of the DGAT1 *K232A* polymorphism.

Breed	Number of animals	Genotype	Genotype frequency (%)	Allele	Allele frequency (%)	H.W.E (χ^2-value)
Kenana	94	*KK*	92.5	*232K*	96.3	0.14NS
		KA	7. 5			
		AA	-	*232A*	3.7	
Butana	91	*KK*	75.8	*232K*	84.6	9.59S
		KA	17.6			
		AA	6.6	*232A*	15.4	

Note: NS: No Significant deviation from Hardy-Weinberg Equilibrium; S: Significant deviation from Hardy-Weinberg Equilibrium.

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. The *232K* allele is fixed in Sahiwal, Rathi, Deoni, Tharparkar, Red Kandhari and Punganur Indian *Bos indicus* breeds [14], Indian Nellore cattle [15], Brazilian Nellore and Guzerat cattle. In the Holstein Friesian breed, frequencies of DGAT1 alleles differed considerably between populations. Thaller et al. (2003) and Rahmatalla et al. (2008) 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 Strzałkowska 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. The frequency of the Lysine variant was lower (12%) in Swedish Holstein cows.

In different studied populations for several dairy cattle breeds, including Holstein Friesian [16-18], Jersey, Ayrshire, and Angeln dairy cattle, 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.

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 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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