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Insertion/deletion polymorphism of the sterol regulatory element-binding protein 1 gene in different cattle breeds

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Abstract: In the present study, an 84-bp insertion/deletion polymorphism of the sterol regulatory element-binding protein 1 (*SREBP1*) gene was investigated. The analysis included 6 Polish cattle herds consisting of beef (Limousin, Angus, Charolais), dairy (Holstein-Friesian, Jersey) and dual-purpose (Montbeliard) breeds. The polymorphism was found only in Limousin and Montbeliard animals, in which both the short (*S*)- and long (*L*)-type alleles occurred. The genotype distribution in Montbeliard cattle was as follows: 0.038, 0.406, and 0.556 for *SS*, *LS*, and *LL*, respectively. To the author's knowledge, this study was the first to investigate the above-mentioned polymorphic site in the Montbeliard and Jersey cattle populations. As far as SREBP1 function is concerned, the examined locus seems to be a very interesting object for further investigations, such as association studies for fatty acid content and composition in cattle milk.

Key words: Cattle, milk, fatty acids, sterol regulatory element-binding protein 1

Sterol regulatory element-binding protein 1 (SREBP1) is the main transcription factor linked to lipogenesis regulation. SREBP1 regulates the expression of the *FASN* and *ACACA* genes coding for the 2 major lipogenic enzymes involved in de novo fatty acid synthesis, fatty acid synthase and acetyl-CoA carboxylase, respectively (1). The SREBP1 pathway plays a key role in the regulation of milk fat synthesis, and thus the variation within the *SREBP1* gene may influence fat content and especially fatty acid composition in bovine milk and meat (2). Hoashi et al. (3) determined the complete sequence of this gene. They discovered an insertion/deletion (ins/del) of 84 bp in intron 5 of the *SREBP1* gene. The short (*S*)- and long (*L*)-type alleles were identified. Recently, several studies have indicated an association between this ins/del polymorphism and fatty acid composition in the milk and meat of cattle (2,4–6). The aim of this study was to analyze the genotype distribution of the above-mentioned polymorphism in cattle populations of different breeds.

The study included a total of 662 individuals of the following breeds: Montbeliard, Limousin, Holstein-Friesian, Angus, Jersey, and Charolais. DNA was extracted from whole peripheral blood, collected from the jugular vein into test tubes containing an anticoagulant (K_3EDTA) using the MasterPure DNA Purification Kit for Blood (Epicentre Biotechnology). The primers used for amplification were those described previously (7). PCR amplifications were performed in a 10-µL volume of reaction mixture containing: approximately 60 ng of genomic DNA, 15 pmol of each primer, 1X Taq DNA polymerase buffer with $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.4 U of Taq DNA polymerase, and nuclease-free deionized water up to 10 µL. Amplifications were carried out according to the following temperature profile: initial denaturation at 94 °C for 5 min, followed by 35 cycles (denaturation at 94 °C for 30 s, primer annealing at 56 °C for 45 s, extension at 72 °C for 45 s) and final extension at 72 °C for 5 min. PCR products were separated in a 1.5% agarose gel in 1X TBE buffer and stained with ethidium bromide. After electrophoresis, DNA bands were visualized under UV light and photographed. Two alleles (*L* and *S*, corresponding to bands of 492 and 408 bp, respectively) and 3 genotypes (*LL*, *LS*, and *SS*) were identified.

The analysis included 6 Polish cattle herds consisting of beef (Limousin, Angus, Charolais), dairy (Holstein-Friesian, Jersey), and dual-purpose (Montbeliard) breeds. Allele and genotype frequencies obtained in the present study are given in the Table. There was no polymorphism in the *SREBP1* locus in dairy cattle populations. All Holstein-Friesian and Jersey individuals were *LL* homozygotes. Kaneda et al. (8) also reported monomorphism in

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Table. Allele and genotype frequencies of the 84-bp insertion/deletion in intron 5 of the *SREBP1* gene in different cattle breeds.

Breed	n	Genotype			Allele	
		SS	LS	LL	S	L
Montbeliard	187	0.038	0.406	0.556	0.241	0.759
Limousin	158	0.126	0.418	0.456	0.335	0.665
Holstein-Friesian	175	-	-	1.000	-	1.000
Angus	49	-	-	1.000	-	1.000
Jersey	50	-	-	1.000	-	1.000
Charolais	43	-	-	1.000	-	1.000

Holstein-Friesian cows. The only study to indicate genetic variation in dairy cattle was presented by Conte et al. (7), who estimated the genotype frequencies in the Italian Brown breed at 0.04, 0.24, and 0.72 for the *SS*, *LS*, and *LL* genotypes, respectively.

All Charolais and Angus cows, investigated in the present study, were *LL* homozygotes. Bhuiyan et al. (5) showed the absence of the *S* allele in the Angus breed, whereas Kaneda et al. (8) indicated its occurrence with a frequency of 0.033. Han et al. (6) found that the frequency of the *S* allele in the Angus and Charolais crossbred was also marginal (0.01), with the absence of the *SS* genotype. The estimated frequency of the *S* allele in Limousin cattle was 0.335, whereas the value reported by Bhuiyan et al. (5) was considerably lower (0.22).

The 84-bp in/del polymorphism in *SREBP1* was deeply investigated in Asian beef cattle breeds, especially in Japanese Black (3,8–10) and Hanwoo, also known as Korean cattle (4,7). The *SS* genotype frequency in Japanese Black varied from 0.07 (9) to 0.27 (10), whereas the heterozygosity level ranged between 0.47 (9) and 0.72 (10). The remaining studied Asian cattle breeds were Brahman, Red Chittagong (5), Japanese Brown, and Mongolian (8), with *S* allele frequencies of 0.00, 0.05, 0.42, and 0.12, respectively. Of the European beef cattle breeds, only Fleckvieh was comprehensively investigated and the estimated *S* allele frequency was 0.08 (4).

Kaneda et al. (8) found a trend in the frequency of the *S* allele in cattle breeds of different origins. The respective values were 0.450 for Japanese Black, 0.117–0.417 for other Asian cattle breeds, and 0.000–0.033 in European and Indian populations, which shows that the 84-bp in/del polymorphism in *SREBP1* could derive from Asian breeds. Nevertheless, this study did not confirm the above-mentioned trend due to the high frequency of allele *S* observed in the 2 different European breeds (0.241 and 0.335 for Montbeliard and Limousin, respectively).

To the author's knowledge, this study was the first to investigate the 84-bp ins/del polymorphism in *SREBP1* in the Montbeliard and Jersey cattle populations. Of the 6 breeds included, variation occurred only in Montbeliard and Limousin cows, whereas the Holstein-Friesian, Jersey, Charolais, and Angus animals were all *LL* homozygotes. As far as SREBP1 function is concerned, the examined locus seems to be a very interesting object for further investigations, such as association studies for fatty acid content and composition in cattle milk.

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