



Polymorphism in the *SREBP-1* gene is associated with milk production traits of Jersey cattle

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Sterol regulatory element-binding protein-1 (*SREBP-1*) is a very important transcription factor involved in the regulation of fat metabolism. It belongs to the family of basic helix-loop-helix leucine zipper transcription factors that plays a crucial role in maintaining energy balance, adipocyte differentiation, and biosynthesis of fatty acids and cholesterol (Brown and Goldstein 1997, Bhuiyan *et al.* 2009). *SREBP-1* upregulates the expression of more than 30 genes required for the process of lipid fraction synthesis that includes fatty acids, cholesterol, glycerophospholipids and triacylglycerols (Horton 2002).

Because of the *SREBP-1* functions, it is thought that the polymorphism of the *SREBP-1* gene could have an impact on a variety of production traits in cattle. In this respect, the most interesting seems to be an 84-bp insertion/deletion polymorphism in intron 5 of the gene reported by Hoashi *et al.* (2007). This polymorphism has been investigated before in numerous cattle breeds including those typical and widely bred such as Holstein-Friesian or Angus as well as rare endogenous breeds (Huang *et al.* 2011, Kaneda *et al.* 2011, Öztapak *et al.* 2013, Proskura 2014). Additionally, potential associations of the *SREBP-1* gene polymorphism with multiple milk production traits have been investigated and confirmed in several cattle breeds (Hoashi *et al.* 2007, Bhuiyan *et al.* 2009, Conte *et al.* 2010, Huang *et al.* 2011, Matsushashi *et al.* 2011, Cecchinato *et al.* 2012).

The aim of this study was to analyze the association between the 84-bp insertion/deletion polymorphism of the *SREBP-1* gene and milk production traits in Jersey cattle.

Jersey cows (234) from 2 herds in tie-stall barns

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located in the Greater Poland Province, were used. The cows were daughters of 43 sires. The feeding was based on total mixed ration (TMR), mainly composed of maize silage, grass haylage, maize cereals, oats cereals, soybean meal and mineral-vitamin mixture. Full data regarding the nutrition were given in the previous study (Proskura *et al.* 2016).

DNA was isolated from whole peripheral blood using the MasterPure™ DNA Purification Kit for Blood (Epicentre, Madison, Wisconsin, USA). The primers for amplification were used as described previously (Conte *et al.* 2010). PCR amplifications were performed according to the protocol described before (Proskura 2014). The PCR products were separated on a 1.5% agarose gel stained with ethidium bromide.

Statistical analyses were performed using the appropriate R packages (R Core Team 2015). An additive relationship matrix was constructed based on the three-generation pedigree using the kinship2 R package (Therneau *et al.* 2014). The following linear model was constructed and estimated using the *lmekin* function in the *coxme* R package (Therneau 2015):

$$Y = \mu + G + H + YS + \beta_1A + \beta_2L + \alpha + e$$

where, Y, phenotypic value of each trait; μ , overall mean; G, fixed effect corresponding to the genotype of the polymorphisms; H, fixed effect of herd; LS, fixed effect of the year-season of calving; β_1A , regression coefficient for the age of cow; β_2L , regression coefficient for lactation length; α , random polygenic component accounting for all known pedigree relationships; and e is a random residual. In the analyses performed simultaneously for all 3 lactations, the fixed effect of lactation was also included.

As a result, 2 alleles were observed, i.e. L (492 base pairs) and S (408 base pairs) combined into the LL and LS genotypes with the frequencies of 0.9 and 0.1, respectively. This is the first study that shows the presence of the S allele in Jersey cattle and the second one that reports its presence in the European breed of dairy cattle. The S allele was minor in the studied population and this was in agreement with

Table 1. The association of an 84-bp insertion/deletion in intron 5 of the *SREBP-1* gene with milk production traits of Jersey cows.

Lactation	Trait	Total (\pm SD)	Genotype		P
			LL (\pm SD)	LS (\pm SD)	
I	MY [kg]	5710.29 \pm 906.87	5691.25 \pm 914.61	5913.95 \pm 812.26	NS
	FY [kg]	290.91 \pm 43.26	291.6 \pm 44.11	283.55 \pm 32.71	NS
	FP [%]	5.12 \pm 0.54	5.16 \pm 0.54	4.77 \pm 0.42	0.031
	PY [kg]	218.82 \pm 33.27	219.04 \pm 33.44	216.45 \pm 32.03	NS
	PP [%]	3.87 \pm 0.27	3.88 \pm 0.27	3.73 \pm 0.15	0.048
II	MY [kg]	6458.39 \pm 929.43	6441.39 \pm 928.72	6734.63 \pm 958.47	NS
	FY [kg]	334.3 \pm 48.4	334.19 \pm 48.21	336 \pm 54.86	NS
	FP [%]	5.21 \pm 0.58	5.22 \pm 0.57	5.02 \pm 0.73	NS
	PY [kg]	253.35 \pm 35.63	253.32 \pm 36.01	253.75 \pm 30.81	NS
	PP [%]	3.92 \pm 0.24	3.93 \pm 0.24	3.78 \pm 0.15	0.04
III	MY [kg]	6492.11 \pm 1059.56	6507.36 \pm 1072.25	6266.4 \pm 917.43	NS
	FY [kg]	337.29 \pm 61.29	338.78 \pm 62.32	315.2 \pm 41.58	NS
	FP [%]	5.21 \pm 0.53	5.22 \pm 0.54	5.06 \pm 0.43	NS
	PY [kg]	255.06 \pm 43.59	256.42 \pm 44.06	235 \pm 33	NS
	PP [%]	3.93 \pm 0.24	3.94 \pm 0.23	3.76 \pm 0.23	0.0075
I-III	MY [kg]	6076.15 \pm 1014	6069.03 \pm 1022.71	6166.3 \pm 905.55	NS
	FY [kg]	312.31 \pm 53.19	313.2 \pm 53.72	301.06 \pm 45.14	NS
	FP [%]	5.16 \pm 0.55	5.19 \pm 0.55	4.87 \pm 0.51	0.03
	PY [kg]	235.73 \pm 39.98	236.32 \pm 40.34	228.3 \pm 34.78	NS
	PP [%]	3.9 \pm 0.25	3.91 \pm 0.26	3.74 \pm 0.16	0.026

MY, milk yield; FY, fat yield; FP, fat percentage; PY, protein yield; PP, protein percentage; NS, non-significant

all previous studies carried out on various cattle breeds. A low frequency of this allele seems to be especially typical of dairy cattle breeds. The highest frequency of the *S* allele observed in dairy cattle was 0.16 in Brown Swiss (Conte *et al.* 2010); while in beef cattle, the frequency was up to 0.45 in Japanese Black (Kaneda *et al.* 2011). In Holstein-Friesians, the *S* allele has not yet been found (Huang *et al.* 2011, Kaneda *et al.* 2011, Proskura 2014) and this may be a result of especially high selection pressure in this breed aimed at increasing milk production.

Our study indicated a significant association of the *SREBP-1* gene polymorphism with milk fat percentage, which is not surprising considering the role of *SREBP-1* in lipid synthesis. The *LS* genotype was linked to a lower fat content in the first lactation and in the first three lactations analyzed together (Table 1). Moreover, the insertion/deletion was associated with milk fat percentage in the first three lactations analyzed both individually and together. In all cases, the *LS* genotype was linked to lower protein content.

A search on The Animal QTL database (Hu *et al.* 2013) indicated that the bovine *SREBP-1* gene is located within the QTL region for milk protein percentage reported by Bennewitz *et al.* (2004) and the QTL for milk fat yield reported by Boichard *et al.* (2003). It was partly in agreement with our study, in which we found the association of the *SREBP-1* polymorphism with milk protein percentage and milk fat percentage, but not with milk fat yield. Because

SREBP-1 plays a pivotal role in lipogenesis (Horton 2002), there is no doubt that any alteration of its functionality may affect numerous traits, e.g. milk composition, and milk lipid fraction in particular. The polymorphism analyzed in this study were located in an intronic region and there was no proof confirming or indicating the way in which it may affect *SREBP-1*. Hoashi *et al.* (2007) suggested that the investigated insertion/deletion may influence the *SREBP-1* level, but did not provide experimental confirmation. Nevertheless, numerous studies had indicated the associations of the polymorphism with production traits in beef and dairy cattle (Hoashi *et al.* 2007, Bhuiyan *et al.* 2009, Conte *et al.* 2010, Huang *et al.* 2011, Matsushashi *et al.* 2011, Cecchinato *et al.* 2012).

This is the first report on the significant associations of the 84-bp insertion/deletion polymorphism in the *SREBP-1* gene with the milk production traits in Jersey cows. The results suggest that the *S* allele might be considered unfavorable in dairy cattle breeding programs. Nevertheless, the lack of *SS* animals in the investigated population did not allow us to draw strong conclusions.

SUMMARY

The sterol regulatory element-binding protein-1 (*SREBP-1*) gene has been indicated to be linked to milk production traits in cattle, especially to milk fat yield and fatty acid composition. The transcription factor encoded by this gene is involved in the regulation of fat metabolism, so it is

supposed that the variability of the *SREBP-1* gene could influence these traits. The aim of this study was to analyze the association between the 84-bp insertion/deletion polymorphism of the *SREBP-1* gene and milk production traits in Jersey cattle. The study found significant associations of the *SREBP-1* gene polymorphism with fat content in the first lactation and protein content in the first three lactations analyzed both individually and together. In all cases, the *LL* genotype was linked to the higher value of the analyzed traits.

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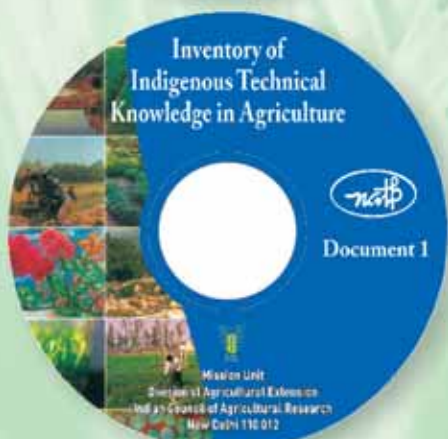
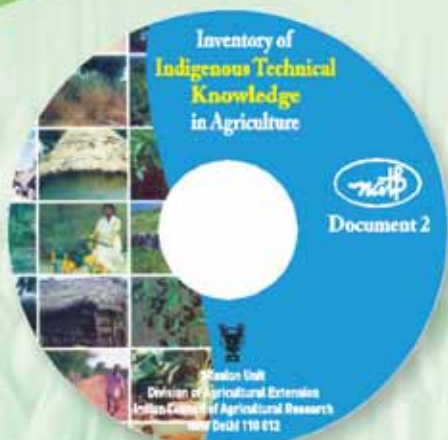
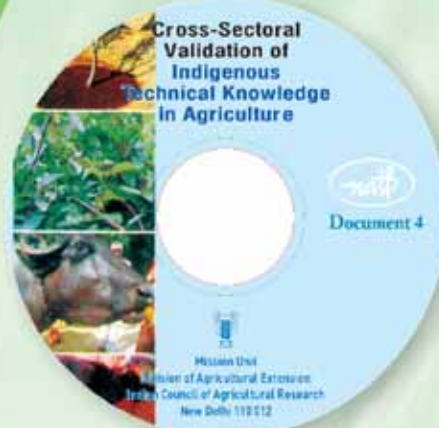
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