

SCD1 SNP IN RELATION TO BREEDING VALUE OF MILK PRODUCTION TRAITS IN POLISH HOLSTEIN-FRIESIAN COWS

Hanna Kulig¹, Inga Kowalewska-Łuczak¹, Kacper Żukowski²,
Melania Kunicka¹

¹West Pomeranian University of Technology, Szczecin, Poland, Department of Genetics and Animal Breeding

²National Research Institute of Animal Production, Balice, Poland, Department of Animal Genetics and Animal Breeding

Abstract. The aim of this study was to investigate associations between *SCD1* polymorphism and the estimated breeding value of milk production traits (milk, fat and protein yield, kg; fat and protein content, %) in the Polish Holstein-Friesian cows' herd. The genotype and allele frequencies for *g.10329C>T* SNP in exon 5 of *SCD1* gene were estimated in the examined herd. Statistical analysis revealed that studied polymorphism significantly affected the breeding value of protein content in milk ($P \leq 0.05$). The results indicated that selection for the *SCD1-TT* individuals might contribute to increased protein content in milk in the Polish Holstein-Friesian cows. Further studies are needed to confirm these results.

Key words: breeding value, cattle, single nucleotide polymorphism

INTRODUCTION

Stearoyl-CoA desaturase (SCD) is an endoplasmic reticulum enzyme. It is involved in conversion of saturated fatty acids into monounsaturated fatty acids by introducing double bond in the delta-9 position in numerous fatty acyl-CoA substrates. The most preferred substrate of the SCD is a stearic acid (C18:0). Products

Corresponding author – Adres do korespondencji: dr Hanna Kulig, West Pomeranian University of Technology, Szczecin, Department of Genetics and Animal Breeding, ul. Doktora Judyma 6, 71-466 Szczecin, Poland, e-mail: hanna.kulig@zut.edu.pl

resulting from the activity of the of the SCD serve as substrates for synthesis of different lipids, such as phospholipids or triglycerides [Ntambi and Miyazaki 2004]. The SCD plays also a role in the endogenous synthesis of the conjugated linoleic acid (CLA) in ruminants. In addition to the diet, breed and the degree of biohydrogenation of unsaturated fatty acids (UFA) in the rumen, the SCD activity in mammary gland affects the proportion of UFA in bovine milk [Jacobs et al. 2011]. Moreover, a role of the SCD-1 in regulation of fatty acid oxidation is suggested [Dobrzyn et al. 2004]. The SCD is expressed in several tissues, mainly in the adipose tissue and liver. Its expression is controlled, among others, by sterol regulatory element-binding protein (SREBP-1c), liver X receptor (LXR), PPARs, insulin and leptin [Paton and Ntambi 2009]. It was suggested that the SCD-1 protein plays an important role in the human metabolic diseases, e.g. morbid obesity and insulin resistance [García-Serrano et al. 2011].

The gene encoding SCD was mapped to bovine chromosome 26 [Campbell et al. 2001], where some QTLs for fat yield and other milk traits have been also identified. Some of the SNPs within the *SCD1* were significantly associated with fatty acid composition in carcass fat tissue and in milk [Taniguchi et al. 2004, Moioli et al. 2007].

The effect of *SCD1* genotypes on breeding value for milk production traits in cattle is very scant. Therefore, the aim of this study was to establish possible associations between the *SCD1* genotypes and breeding values for milk production traits in Polish Holstein-Friesian cows.

MATERIAL AND METHODS

The study covered 975 Polish Holstein-Friesian (Black & White strain) cows belonging to one herd kept on a farm located in the western region in Poland. Animals were kept in identical environmental conditions and were fed a standard diets. Genomic DNA was extracted from blood using MasterPure™ Genomic DNA Purification Kit (Epicentre® Biotechnologies).

The *g.10329C>T* SNP in exon 5 of the *SCD1* gene (GeneBank acc. no. AY24-1932) described by Taniguchi et al. [2004] was analysed. The *SCD1* genotyping was performed according to Taniguchi et al. [2004].

The PCR was performed in a total volume of 20 µL containing 50-80 ng DNA, 20 mM Taq polymerase buffer, 2 mM MgCl₂, 10 pmol each primer, 200 mM each dNTP, and 0.5 U Taq DNA polymerase. The amplicons were digested with *SatI* restriction endonuclease. The obtained restriction fragments were separated on 3% agarose gels with ethidium bromide (0.5 µg/mL) in the presence of an

appropriate DNA standard, and described using the Vilber Lourmat software for photodocumentation of electrophoretic separation and image storage.

Next, a statistical analysis of associations between genotypes and the estimated breeding value (EBV) for milk yield – MY (kg), fat yield – FY (kg), fat content – FC (%), protein yield – PY (kg), protein content – PC (%) and index – I (kg) as a fat yield and the doubled protein yield breeding value (kg). EBV data came from official electronic documentation of the herd. Evaluations were performed by The National Research Institute of Animal Production in Balice near Krakow (Poland).

The association analysis was performed as a regression of EBV for MY, FY, FC, PY, PC and I of *SCD1* genotypes using MIXED procedure implemented in SAS (SAS v. 9.3). The following linear model was applied:

$$y_i = \mu + b_i + \varepsilon_i$$

where:

y_i – predicted breeding value of cow,

μ – overall mean,

b_i – the fixed effect of SNP *i*-th genotype for *g.10329C>T* (*i* = 1, 2, 3) and

ε – an error.

The associations were tested by using the t-test with the Bonferroni correction for multiple testing. The differences between the means were compared by the Duncan's multiple range tests with the LSMEANS for MIXED statement.

RESULTS

In the studied cows' herd, all possible *SCD1* genotypes were identified. The frequencies of the analysed genotypes and alleles were as follows: *CC* – 0.54, *CT* – 0.40, *TT* – 0.06, *C* – 0.74, *T* – 0.26.

Means of EBV for milk production traits in relation to the analysed genotypes are given in the Table 1. Statistical analysis revealed that the genotypes were associated with EBV for protein content. It was confirmed after Bonferroni correction. The *TT* cows were characterized by significantly ($P \leq 0.01$) higher EBV for this trait than the *CC* cows; the difference was 0.03 % in average. This SNP also significantly ($P \leq 0.05$) influenced EBV for fat content, but after Bonferroni correction this trend was not maintained.

In terms of estimated breeding value for milk, fat and protein yields, the differences between the respective *SCD1* genotypes were not statistically significant.

However, it was found that the *CT* genotype cows were characterised by the highest milk, protein, and fat yields.

Table 1 Means with standard error of estimated breeding values for milk production traits in cows with different *SCD1* genotypes

Tabela 1 Średnie i błąd standardowy oszacowanej wartości hodowlanej dla cech użytkowości mlecznej u krów z różnymi genotypami *SCD1*

Trait Cecha	Genotype – Genotyp			Significance – Istotność	
	<i>TT</i> (n = 60)	<i>CT</i> (n = 391)	<i>CC</i> (n = 524)	P-value	P-value <i>Bonf</i>
MY	216.98 (48.94)	313.05 (19.17)	297.71 (16.56)	0.18	0.54
FY	9.74 (1.63)	10.79 (0.64)	8.99 (0.55)	0.10	0.30
FC	0.01 (0.02) ^A	-0.03 (0.01)	-0.05 (0.01) ^B	0.04	0.12
PY	9.03 (1.47)	11.19 (0.58)	9.64 (0.50)	0.09	0.27
PC	0.03 (0.01) ^A	0.01 (0.00)	0.00 (0.00) ^B	0.00	0.01
I	27.79 (4.31)	33.18 (1.69)	28.26 (1.46)	0.07	0.21

MY – milk yield (kg), FY – fat yield (kg), FC – fat content (%), PY – protein yield (kg), PC – protein content (%), I – index (kg), means in rows marked with the different letter differ significantly at $P \leq 0.05$ (small letters) and at $P \leq 0.01$ (big letters), means with the same letter are not significantly different.

MY – wydajność mleka (kg), FY – wydajność tłuszczu (kg), FC – zawartość tłuszczu (%), PY – wydajność białka (kg), PC – zawartość białka (%), I – indeks (kg), średnie wartości w rzędach oznaczone różnymi literami różnią się między sobą istotnie $P \leq 0.01$.

DISCUSSION

Genetic polymorphisms significantly associated with economically important traits of milk cattle may be helpful to improve the efficiency of traditional selection methods. Studies reporting analysis of SNPs that may explain the mechanisms underlying genetic variation of these traits are still conducted. Statistically significant associations between polymorphisms in some of genes and milk production traits as well as milk fat related traits have been found [Zhang et al. 2009, Manga and Řiřha 2011, Mao et al. 2012, Matsumoto et al. 2012].

The *SCD1* is proposed as candidate gene for milk fat traits in cattle. It is supported by their chromosomal localisation and by the physiological function of its protein product [Ogorevc et al. 2009]. Polymorphic sites within the *SCD1* gene were identified and they included three SNPs in exon 5 and several SNPs in 3'UTR [Taniguschi et al. 2004, Jiang et al. 2008]. One of the exonic SNP, analysed in the present study *g.10329C>T*, causes an alanine-to-valine substitution in the polypeptide sequence. Analysis of the *g.10329C>T* allele frequencies showed the C allele to be most frequent (0.315) in the studied Polish Holstein-Friesian cows, which was comparable to the literature data concerning breeds such as Italian Holstein, Canadian Holstein, Japanese Black, Valdostana, Fleckvieh [Taniguschi

et al. 2004, Mele et al. 2007, Moioli et al. 2007, Macciotta et al. 2008, Kgwatalala et al., 2009, Bartoñ et al. 2010, Matsuhashi et al. 2011].

Results in the present study show associations between the *g.10329C>T* genotypes and the breeding value for protein content in Polish Holstein-Friesian cows; statistically significant difference was observed between cows with *TT* and *CC* genotype. The *TT* genotype cows were characterized by a higher fat and protein contents, but the *CT* individuals showed a higher breeding value for milk, fat and protein yields. This general trend observed in the present study confirms results obtained by Komisarek and Dorynek [2009] in Polish Holstein-Friesian bulls and by Alim et al. [2012] in Chinese Holstein cows. Although, Komisarek and Dorynek [2009] showed associations between the *g.10329C>T* genotypes and the breeding value for fat content in milk but it was not confirmed after correction for FDR, while Alim et al. [2012] found significant association between this SNP and milk, fat and protein yields. Moreover, Macciotta et al. [2008] showed a significantly higher daily milk and protein yields for the *TT* genotype, compared with the *CC* and *CT* genotype in Italian Holstein cows.

The *SCD1* SNP was also analysed with regard to milk fatty acid composition. The effect of *g.10329C>T* genotype on desaturation indices and on percentage of some individual fatty acids in milk was reported by Mele et al. [2007], Moioli et al. [2007], Schennink et al. [2008] and Kgwatalala et al. [2009] in Italian Holstein-Friesian, Piedmontese, Valdostana, Dutch Holstein-Friesian and Canadian Holstein cattle. Associations between *SCD1* gene polymorphisms and fatty acid profile were analysed in carcass fat too. The *g.10329C>T* SNP was significantly associated with fatty acid composition of intramuscular fat in Japanese Black [Taniguschi et al. 2004, Matsuhashi et al. 2011], Holstein [Narukami et al. 2011], Simmental [Orrù et al. 2011] and Flekvieh cattle [Bartoñ et al. 2010], and of subcutaneous fat in Flekvieh [Bartoñ et al., 2010] and Canadian beef cattle [Li et al. 2011].

CONCLUSIONS

The results of this study suggest that the *SCD1-TT* genotype animals might be useful in selection toward increasing protein content in milk of the Polish Holstein-Friesian cattle. Nevertheless, further studies are needed to confirm this.

ACKNOWLEDGEMENT

The work was supported by the West Pomeranian University of Technology, Szczecin (Poland).

REFERENCES

- Alim M.A., Fan Y.P., Wu X.P., Xie Y., Zhang Y., Zhang S.L., Sun D.X., Zhang Y., Zhang Q., Liu L., Guo G., 2012. Genetic effects of stearoyl-coenzyme A desaturase (SCD) polymorphism on milk production traits in the Chinese dairy population. *Mol. Biol. Rep.*, 39, 8733–8740.
- Bartoň L., Kott T., Bureš D., Řehák D., Zahradková R., Kottová B., 2010. The polymorphisms of stearoyl-CoA desaturase (*SCD1*) and sterol regulatory element binding protein-1 (*SREBP-1*) genes and their association with the fatty acid profile of muscle and subcutaneous fat in Fleckvieh bulls. *Meat. Sci.*, 85, 15–20.
- Campbell E.M.G., Gallagher D.S., Davis S.K., Taylor J.F., Smith S.B., 2001. Rapid communication: Mapping of the bovine stearoyl-coenzyme A desaturase (SCD) gene to BTA26. *J. Anim. Sci.*, 79, 1954–1955.
- Dobrzyn P., Dobrzyn A., Miyazaki M., Cohen P., Asilmaz E., Hardie D.G., Friedman J.M., Ntambi J.M., 2004. Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. *PNAS*, 101, 6409–6414.
- García-Serrano S., Moreno-Santos I., Garrido-Sánchez L., Gutierrez-Repiso C., García-Almeida J.M., García-Arnés J., Rivas-Marín J., Gallego-Perales J.L., García-Escobar E., Rojo-Martinez G., Tinahones F., Soriguer F., Macías-Gonzalez M., García-Fuentes E., 2011. Stearoyl-CoA Desaturase-1 Is Associated with Insulin Resistance in Morbidly Obese Subjects. *Mol. Med.*, 17, 273–280.
- Jacobs A.A.A., van Baal J., Smits M.A., Taweel H.Z.H., Hendriks W.H., van Vuuren A.M., Dijkstra J., 2011. Effects of feeding rapeseed oil, soybean oil, or linseed oil on stearoyl-CoA desaturase expression in the mammary gland of dairy cows. *J. Dairy Sci.*, 94, 874–87.
- Jiang Z., Michal J.J., Tobey D.J., Daniels T.F., Rule D.C., MacNeil M.D., 2008. Significant associations of stearoyl-CoA desaturase (*SCD1*) gene with fat deposition and composition in skeletal muscle. *Int. J. Biol. Sci.*, 4, 345–351.
- Kgwatalala P.M., Ibeagha-Awemu E.M., Mustafa A.F., Zhao X., 2009. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. *Anim. Genet.*, 40, 609–615.
- Komisarek J., Dorynek Z., 2009. Effect of *ABCG2*, *PPARGC1A*, *OLR1* and *SCD1* gene polymorphism on estimated breeding values for functional and production traits in Polish Holstein-Friesian bulls. *J. Appl. Genet.*, 50, 125–132.
- Li C., Aldai N., Vinsky M., Dugan M.E.R., McAllister T.A., 2011. Association analyses of single nucleotide polymorphisms in bovine stearoyl-CoA desaturase and fatty acid synthase genes with fatty acid composition in commercial cross-bred beef steers. *Anim. Genet.*, 43, 93–97.
- Macciotta N.P.P., Mele M., Conte G., Serra A., Castiglioni B., Chessa S., Buccioni A., Pagnacco G., Secchiari P., 2008. Association Between a Polymorphism at the Stearoyl CoA Desaturase Locus and Milk Production Traits in Italian Holsteins. *J. Dairy Sci.*, 91, 3184–3189.

- Manga I., Říha H., 2011. The DGAT1 gene K232A mutation is associated with milk fat content, milk yield and milk somatic cell count in cattle (Short Communication). Arch. Tierz., 54, 257–263.
- Mao Y.J., Chen R.J., Chang L.L., Chen Y., Ji D.J., Wu X.X., Shi X.K., Wu H.T., Zhang M.R., Yang Z.P., König S., Yang L.G., 2012. Effects of *SCD1*- and *DGAT1*-genes on production traits of Chinese Holstein cows located in the Delta Region of Yangtze River. Liv. Sci., 145, 280–286.
- Matsumoto H., Inada S., Kobayashi E., Abe T., Hasebe H., Sasazaki S., Oyama K., Mannen H., 2012. Identification of SNPs in the *FASN* gene and their effect on fatty acid milk composition in Holstein cattle. Liv. Sci., 144, 281–284.
- Matsuhashi T., Maruyama S., Uemoto Y., Kobayashi N., Mannen H., Abe T., Sakaguchi S., Kobayashi E., 2011. Effects of bovine fatty acid synthase, stearoyl-coenzyme A desaturase, sterol regulatory element-binding protein 1, and growth hormone gene polymorphisms on fatty acid composition and carcass traits in Japanese Black cattle. J. Anim. Sci., 89, 12–22.
- Mele M., Conte G., Castiglioni B., Chessa S., Macciotta N.P.P., Serra A., Buccioni A., Pagnacco G., Secchiari P., 2007. Stearoyl-Coenzyme A Desaturase Gene Polymorphism and Milk Fatty Acid Composition in Italian Holsteins. J. Dairy Sci., 90, 4458–4465.
- Moioli B., Contarini G., Avalli A., Catillo G., Orrù L., De Matteis G., Masoero G., Napolitano F., 2007. Short Communication: Effect of Stearoyl-Coenzyme A Desaturase Polymorphism on Fatty Acid Composition of Milk. J. Dairy Sci., 90, 3553–3558.
- Narukami T., Sasazaki S., Oyama K., Nogi T., Taniguchi M., Mannen H., 2011. Effect of DNA polymorphisms related to fatty acid composition in adipose tissue of Holstein cattle. Anim. Sci. J., 82, 406–411.
- Ntambi, J.M., Miyazaki M., 2004. Regulation of stearoyl-CoA desaturases and role in metabolism. Prog. Lipid Res., 43, 91–104.
- Ogorevc J., Kunej T., Razpet A., Dovc P., 2009. Database of cattle candidate genes and genetic markers for milk production and mastitis. Anim. Genet., 40, 832–851.
- Orrù L., Cifuni G.F., Piasentier E., Corazzin M., Bovolenta S., Moioli B., 2011. Association analyses of single nucleotide polymorphisms in the *LEP* and *SCD1* genes on the fatty acid profile of muscle fat in Simmental bulls. Meat. Sci., 87, 344–348.
- Paton C.M., Ntambi J.M., 2009. Biochemical and physiological function of stearoyl-CoA desaturase. Am. J. Physiol-Endoc M 297, E28–E37, Doi: 10.1152/ajpendo.90897.2008, <http://ajpendo.physiology.org>
- Schennink A., Heck J.M.L., Bovenhuis H., Visker M.H.P.W., Van Valenberg H.J.F., Van Arendonk J.A.M., 2008. Milk fatty acid unsaturation: Genetic parameters and effects of stearoyl-CoA desaturase (*SCD1*) and acyl CoA:diacylglycerol acyltransferase 1 (*DGAT1*). J. Dairy Sci., 91, 2135–2143.
- Taniguchi M., Utsugi T., Oyama K., Mannen H., Kobayashi M., Tanabe Y., Ogino A., Tsuji S., 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. Mamm. Genom., 14, 142–148.

Zhang S., Knight T.J., Reecy J.M., Wheeler T.L., Shackelford S.D., Cundiff L.V., Beitz D.C., 2009. Associations of polymorphisms in the promoter I of bovine acetyl-CoA carboxylase- α gene with beef fatty acid composition. *Anim. Genet.*, 41, 417–20.

SNP W GENIE *SCD1* W POWIĄZANIU Z WARTOŚCIĄ HODOWLANĄ DLA CECH UŻYTKOWOŚCI MLECZNEJ U KRÓW RASY POLSKIEJ HOLSZTYŃSKO-FRYZYJSKIEJ

Streszczenie. Celem badań było zbadanie zależności między polimorfizmem genotypu *SCD1* a oszacowaną wartością hodowlaną dla cech użytkowości mlecznej (wydajności mleka, tłuszczu i białka, kg oraz zawartością tłuszczu i białka w mleku, %) w stadzie krów rasy polskiej holsztyńsko-fryzyjskiej. Oszacowano częstość występowania genotypów i alleli SNP *g.10329C>T* zlokalizowanego w eksonie 5. genu *SCD1*. Analiza statystyczna wykazała, że badany polimorfizm wpływał istotnie na wartość hodowlaną dla zawartości białka ($P \leq 0,01$). Wyniki wskazują, że uwzględnienie w selekcji osobników z genotypem *SCD1-TT* mogłoby przyczynić się do zwiększenia zawartości białka w mleku krów rasy polskiej holsztyńsko-fryzyjskiej. Wymagane jest jednak kontynuowanie badań aby móc zweryfikować uzyskane wyniki.

Słowa kluczowe: bydło, polimorfizm pojedynczego nukleotydu, wartość hodowlana

Accepted for print – Zaakceptowano do druku: 30.01.2013