Animal Science Papers and Reports vol. 31 (2013) no. 4, 273-279
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

SLC27A1 SNPs in relation to breeding value of milk production traits in Polish Holstein-Friesian cows*

Hanna Kulig[1], Inga Kowalewska-Łuczak[1], Kacper Żukowski[2], Melania Kunicka[1], Wojciech Kruszyński[3]**

[1] Department of Genetics and Animal Breeding,
West Pomeranian University of Technology in Szczecin,
Doktora Judyma 6, 71-466 Szczecin, Poland

[2] Department of Animal Genetics and Animal Breeding,
National Research Institute of Animal Production,
Krakowska 1, 32-083 Balice/Kraków, Poland

[3] Department of Genetics, Wrocław University of Environmental and Life Sciences,
Kożuchowska 7, 51-631 Wrocław, Poland

(Received December 5, 2012; accepted September 4, 2013)

The aim of the study was to investigate associations between *SLC27A1* genotypes and estimated breeding value of milk production traits (milk, fat and protein yield, kg; fat and protein content, %) in the Polish Holstein-Friesian cows' herd. Three single nucleotide polymorphisms were genotyped, the *g.14996C>G*. in the exon 3, *g.14791C>T* in exon 4 and *g.14589A>G* in exon 5 of *SLC27A1* gene. The genotype and allele frequencies for each polymorphism and the *SLC27A1* haplotype frequencies were estimated in the examined herd. Significant relations between the *SLC27A1 g.14791C>T* SNP and breeding value for protein content were found. The results indicate that selection for the *SLC27A1-CC* individuals might contribute to increased protein content of milk in Polish Holstein-Friesian cows. Further studies are needed to confirm these results.

KEY WORDS: breeding value / cattle / gene polymorphism / milk traits

*Supported by the West Pomeranian University of Technology in Szczecin, Poland.

**Corresponding author: hanna.kulig@zut.edu.pl

SLC27A1 (solute carrier family 27 member 1) is a member of the fatty acid transport protein family. It is the transmembrane protein that facilitates long chain fatty acid (LCFA) transport across the cytoplasmic membrane. The study conducted on mice purified SLC27A1 protein revealed its long-chain and very long-chain acyl-CoA synthetase activity [Hall *et al*. 2003]. SLC27A1, translocated to the cell membrane from intracellular sites, participates in the insulin-stimulated LCFA influx. It also plays a role as the regulator of Krebs' cycle activity and therefore assists in mitochondrial function [Wiczer and Bernlohr 2009]. SLC27A1 is expressed in various tissues, predominantly in those, which are characterised by rapid fatty acids metabolism. In human and in mice, the highest SLC27A1 mRNA level was found in the adipose tissue, heart, skeletal muscle and brain [Martin *et al*. 2000]. This expression profile was confirmed in the bovine tissues, except for adipose tissue, where SLC27A1 mRNA levels are low [Ordovás *et al*. 2006]. Transcription of the SLC27A1 is controlled by insulin and PPARs (peroxisome proliferators-activated receptors) [Wiczer and Bernlohr 2009]. Some studies in humans and mice suggest that SLC27A1 is involved in the control of energy homeostasis and may play a role e.g. in the pathophysiology of type 2 diabetes [Wu *et al*. 2006]. Moreover an association is suggested between the intronic polymorphism and changes in triglyceride metabolism [Meirhaeghe *et al*. 2000, Gertow *et al*. 2003].

SLC27A1 encoding gene was mapped to bovine chromosome 7, where QTLs for milk production traits have been identified [Ordovás *et al*. 2005, Ogorevc *et al*. 2009]. SNPs were identified within the bovine *SLC27A1,* one of them significantly associated with milk fat yield in Chinese Holstein cattle [Ordovás *et al*. 2008, Lv *et al*. 2011].

There are few reports on *SLC27A1* polymorphisms and their association with economically important traits of cattle. Therefore, the aim of this study was to establish possible associations between the *SLC27A1* genotypes and breeding values for milk production traits in Polish Holstein-Friesian cows.

Material and methods

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

The three SNPs described by Ordovás *et al*. [2008] were analysed: *g.14996C>G* in exon 3, *g.14791C>T* in exon 4 and *g.14589A>G* in the exon 5 (GeneBank acc. no. AAFC03051286). Genotypes were determined by PCR-RFLP method and primers were designed on the basis of the respective sequences shown in Table 1.

Afterwards, the restriction enzymes for SNP identification were matched using the NEBcutter (version 2.0) software tool. The *Sac*II, *Bsa*HI and *Ava*I restriction

Table 1. Outline for amplification the *SLC27A1* gene fragments

SNP	Primer pairs	Amplicon size bp
g.14996C>G	5'-CTGCTCAACGTGAACCTGCG-3' 5'-ACCAGGCTCTTGCCCAACTC-3'	261
g.14791C>T	5'-*CCATCTTCAACCACGACGTG*-3' 5'-*GCTGTGGAGGTCTCCTTCAG*-3'	160
g.14589A>G	5'-*CGACTCTTCTACATCTACACCTC*-3' 5'-*CACAGGATCCTCAACAAGAGCTG*-3'	143

endonucleases were chosen for *g.14996C>G*, *g.14791C>T* and *g.14589A>G* genotyping, respectively. One of the primers was mismatched (mismatch base shown in Table 1 as underlined) in order to create an *Ava*I restriction endonuclease recognition site. Restriction fragments were separated on 3% agarose gels and described using the software for photo-documentation of electrophoretic separation and image storage (VILBER LOURMAT). Haplotypes were predicted for the cows' herd in this study by a PHASE (v2.1) programme. Estimation of frequency for *SLC27A1* haplotypes was carried out using HAPLOVIEW software.

Next, a statistical analysis of associations between genotypes and the estimated breeding value (EBV) for milk yield – MY (kg milk), fat yield – FY (kg fat), fat content – FC (% fat), protein yield – PY (kg protein), protein content – PC (% protein) and index – In (kg) as a fat yield and the doubled protein yield breeding value (kg). The breeding values for cows are expressed as lactation breeding values obtained by summing up breeding values for day 5 to 305. Variances of second and third lactations were standardized to the first lactation variance, and then the mean lactation breeding values are calculated. EBV data came from official electronic documentation of the herd. Evaluations were performed by The National Research Institute of Animal Production in Balice near Crakov (Poland).

An association analysis was carried out as a regression of EBV on MY, FY, FC, PY, PC and In of *SLC27A1* genotypes using MIXED procedure implemented in SAS (SAS v. 9.3). Procedure was also used to check association between the mentioned productive traits and *SLC27A1* haplotype combinations. The following linear model was applied:

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

where:

y_i – predicted breeding value of a cow;

μ – overall mean;

b_i – the fixed effect of SNP i-th genotype for *g.14996C>G*), *g.14791C>T*), *g.14589A>G* (i = 1,2,3) or i-th haplotype combinations for *SLC27A1* (i = 1, 2, 3, ..., 10);

ε_i – error.

The associations were tested by the t- test with the Bonferroni correction for multiple testing. Differences between the means were compared by the Duncan's multiple range tests with the Least Squares Means for MIXED statement.

Results and discussion

The *g.14996C>G* PCR product digestion with *Sac*II enzyme resulted in non-cutting fragment (allele *G*) and the 169 and 92 bp restriction fragments (allele *C*). In case of *g.14791C>T* polymorphism, the PCR product digested with *Bsa*HI enzyme revealed a non-cutting fragment (allele *T*) and cutting fragments of 106 and 54 bp (allele *C*). The *g.14589A>G* PCR product digestion with *Ava*I enzyme resulted in non-cutting fragment (allele *A*) and the 122 and 21 bp restriction fragments (allele *G*).

Table 2. The genotype and allele frequencies of the studied SNPs

SNP	Genotype frequencies		Allele frequencies	
g.14996C>G	*GG*	0.63	*G*	0.80
	CG	0.35	*C*	0.20
	CC	0.02		
g.14791C>T	*TT*	0.36	*T*	0.64
	CT	0.56	*C*	0.36
	CC	0.08		
g.14589A>G	*GG*	0.44	*G*	0.70
	AG	0.52	*A*	0.30
	AA	0.04		

In the studied herd, all possible *SLC27A1* genotypes were identified. The frequencies of the analysed genotypes and alleles are presented in Table 2. Nineteen *SLC27A1* haplotype combinations were detected in the herd (Tab. 3).

Statistical analysis revealed that the *SLC27A1-2* genotypes were associated with breeding value for milk protein content which was confirmed after Bonferroni correction (Tab. 4). The *TT* cows showed a significantly lower breeding value for protein percentage in milk compared to the *CC* ($P \leq 0.01$) individuals, the difference amounting to 0.04. As regards the other *SLC27A1* polymorphisms, no significant differences were found between genotypes and analysed milk production traits of the cows studied. Moreover, no associations were found in this study between the *SLC27A1* haplotype combination and the traits analysed (data not shown).

Genetic polymorphisms significantly associated with economically important traits of cattle are useful in explaining the mechanisms underlying their genetic variation. They may be very helpful in improving the accuracy and efficiency of traditional selection methods. Therefore, association studies are still continued. Studies reporting analysis of variants of genes participating in fatty acids binding, transport, and metabolism in relation to production traits in cattle include *FASN* (fatty acid synthase), *DGAT1* (diacylglycerol O-acyltransferase 1), *SCD1* (stearoyl-

Table 3. The *SLC27A1* haplotype and haplotype combination frequencies

Haplotype	Haplotype frequency	Haplotype combination	Percent of haplotype combination
1(GTG)	0.564	1/1(GGTTGG)	26.36
2(CCA)	0.162	2/1(CGCTAG)	24.10
3(GCG)	0.117	3/1(GGCTGG)	14.46
4(GCA)	0.075	4/1(GGCTAG)	13.95
5(GTA)	0.051	5/1(GGTTAG)	6.87
6(CTG)	0.015	2/3(CGCCAG)	4.51
7(CTA)	0.010	6/1(CGTTGG)	1.64
8(CCG)	0.006	7/1(CGTTAG)	1.13
		8/1(CGCTGG)	1.03
		2/4(CGCCAA)	1.03
		2/5(CGCTAA)	0.92
		4/3(GGCCAG)	0.82
		2/2(CCCCAA)	0.72
		2/7(CCCTAA)	0.72
		3/3(GGCCGG)	0.62
		2/6(CCCTAG)	0.51
		4/5(GGCTAA)	0.31
		2/8(CCCCAG)	0.21
		8/3(CGCCGG)	0.10

Table 4. Means with standard errors of estimated breeding values for milk production traits in cows of different *g.14791C>T* genotypes

Trait	Genotype			Significance	
	TT (n = 351)	*CT* (n = 546)	*CC* (n = 78)	P-value	P-value Bonf
MY	295.75 (20.24)	310.21 (16.23)	233.82 (42.93)	0.25	0.75
FY	9.31 (0.67)	10.08 (0.54)	9.54 (1.43)	0.66	1
FC	-0.04 (0.01)	-0.04 (0.01)	0.00 (0.02)	0.21	0.63
PY	9.39 (0.61)	10.84 (0.49)	9.70 (1.29)	0.17	0.51
PC	-0.01 (0.01)^{B}	0.01 (0.01)^{AB}	0.03 (0.01)^{A}	0.00	0.01
In	28.09 (1.78)	31.75 (1.43)	28.93 (3.78)	0.26	0.78

MY – milk yield (kg); FY – fat yield (kg); FC – fat content (%); PY – protein yield (kg); PC –protein content (%); In – index (kg). ^{AB}Means in row marked with different superscripts differs significantly at P≤0.01.

CoA desaturase 1), *ACACA* (acetyl-CoA carboxylase alpha). Significant associations between polymorphisms in some of these genes and milk production traits as well as milk fat related traits have already been found [Zhang *et al*. 2009, Komisarek *et al*. 2011, Mao *et al*. 2012, Matsumoto *et al*. 2012].

The *SLC27A1* could be proposed as candidate gene for milk fat traits in cattle. It is supported by their chromosomal localisation and by the physiological function of their protein products [Ordovás *et al*. 2008, Ogorevc *et al*. 2009].

Ordovás *et al*. [2008] identified SNPs within the bovine *SLC27A1* gene, one of them located in the promoter region, six in coding exons and seven in introns. In case of three exonic SNPs, the *g.14996C>G*, *g.14791C>T* and *g.14589A>G*, the *G*, *T* and *G* alleles, respectively, were most frequent in the Holstein-Friesian, Asturiana de los Valles and Menorguina breeds. It was comparable to the data of the Polish Holstein-Friesian herd presented here. Although all identified by Ordovás *et al*. [2008] exonic SNPs were synonymous, it is considered, that type of mutation can effect gene function, e.g. by modulating splicing, translation efficacy [Woolfe *et al*. 2010].

Results obtained in this study demonstrate that there are significant associations between the *g.14791C>T* polymorphism and EBV for milk protein content in Polish Holstein-Friesian cows. It is worth mentioning that the *SLC27A1* gene is located within a region containing QTLs for milk protein content [Mosig *et al*. 2001], so the SNPs analysed might be linked to the functional polymorphisms for this trait. Ordovás *et al*. [2008] analysed the potential association with estimated breeding value for milk fat content, but they found no associations between the SNPs and this trait in the Holstein-Friesian population. Associations between *SLC27A1* gene polymorphisms and milk production traits were analysed in Chinese Holstein cattle. In that study, Lv *et al*. [2011] found associations between *g.14791C>T* SNP (named by authors as *112T>C* according to GeneBank acc. no. NM_001033625.2) and EBV for milk yield. The *CC* animals were characterized by significantly higher ($P \leq 0.05$) value of milk compared with the *CT* and *TT* individuals.

SNPs were also identified in the chicken *SLC27A1* gene and some of them (as diplotypes) have been associated with carcass traits, such as live weight, carcass weight, and semi-eviscerated weight [Wang *et al*. 2010]. Moreover, there was a report on the porcine *SLC27A1* gene polymorphism.

The results of this study suggest that the *SLC27A1-CC* 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.

REFERENCES

1. GERTOW K., SKOGLUND-ANDERSSON C., ERIKSSON P., BOQUIST S., ORTH-GOMER K., SCHENCK-GUSTAFSSON K., HAMSTEN A., FISHER R.M., 2003 – A common polymorphism in the fatty acid transport protein-1 gene associated with elevated post-prandial lipaemia and alterations in LDL particle size distribution. ***Atherosclerosis*** 167, 265-273.
2. HALL A.M., SMITH A.J., BERNLOHR D.A., 2003 – Purified Murine Fatty Acid Transport Protein 1. ***Journal of Biological Chemistry*** 278, 43008-43013.
3. KOMISAREK J., MICHALAK A., WALENDOWSKA A., 2011 – The effects of polymorphisms in *DGAT1*, *GH* and *GHR* genes on reproduction and production traits in Jersey cows. ***Animal Science Papers and Reports*** 29, 29-36.
4. LV Y., WEI C., ZHAN L., LU G., LIU K., DU L., 2011 – Association between polymorphisms in the *SLC27A1* gene and milk production traits in Chinese Holstein cattle. ***Animal Biotechnology*** 22, 1-6.

5. 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., KONIG 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. ***Livestock Science*** 145, 280-286.
6. MARTIN G., NEMOTO M., GELMAN L., GEFFROY S., NAJIB J., FRUCHART J.C., ROEVENS P., DE MARTINVILLE B., DEEB S., AUWERX J., 2000 – The Human Fatty Acid Transport Protein-1 (*SLC27A1*; *FATP-1*) cDNA and Gene: Organization, Chromosomal Localization, and Expression. ***Genomics*** 66, 296-304.
7. 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. ***Livestock Science*** 144, 281-284.
8. MEIRHAEGHE A., MARTIN G., NEMOTO M., DEEB S., COTTEL D., AUWERX J., AMOUYEL P., HELBECQUE N., 2000 – Intronic polymorphism in the fatty acid transport protein 1 gene is associated with increased plasma triglyceride levels in a French population. ***Arteriosclerosis, Thrombosis, and Vascular Biology*** 20, 1330-1334.
9. MOSIG M.O., LIPKIN E., KHUTORESKAYA G., TCHOURZYNA E., SOLLER M., FRIEDMANN A., 2003 – A whole genome scan for QTL affecting milk protein percentage in Israeli-Holstein cattle by means of selective milk DNA pooling in a daughter design, using an adjusted false discovery rate criterion. ***Genetics*** 157, 1683–1698.
10. OGOREVC J., KUNEJ T., RAZPET A., DOVC P., 2009 – Database of cattle candidate genes and genetic markers for milk production and mastitis. ***Animal Genetics*** 40, 832-851.
11. ORDOVÁS L., ROY R., ZARAGOZA P., HAYES H., EGGEN A., RODELLAR C., 2005 – Assignment of the solute carrier family 27 member 1 (*SLC27A1*) gene to bovine chromosome 7. ***Animal Genetics*** 36, 352-363.
12. ORDOVÁS L., ROY R., ZARAGOZA P., RODELLAR C., 2006 – Structural and functional characterization of the bovine solute carrier family 27 member 1 (*SLC27A1*) gene. ***Cytogenetic and Genome Research*** 115, 115-122.
13. ORDOVÁS L., ZARAGOZA P., ALTARRIBA J., RODELLAR C., 2008 – Identification of 14 new single nucleotide polymorphisms in the bovine *SLC27A1* gene and evaluation of their association with milk fat content. ***Journal of Dairy Research*** 75, 129-134.
14. WANG Y., ZHU Q., ZHAO X.-L., YAO Y.G., LIU Y.P., 2010 – Association of *FATP1* gene polymorphisms with chicken carcass traits in Chinese meat-type quality chicken populations. ***Molecular Biology Reports*** 37, 3683-3690.
15. WICZER B.M., BERNLOHR D.A., 2009 – A novel role for fatty acid transport protein 1 in the regulation of tricarboxylic acid cycle and mitochondrial function in 3T3-L1 adipocytes, ***Journal of Lipid Research*** 50, 2502-2513.
16. WOOLFE A., MULLIKIN J.C., ELNITSKI L., 2010 – Genomic features defining exonic variants that modulate splicing. ***Genome Biology*** 11, R20, doi:10.1186/gb-2010-11-2-r20, http://genomebiology.com.
17. WU Q., ORTEGON A.M., TSANG B., DOEGE H., FEINGOLD K.R., STAHL A., 2006 – FATP1 Is an Insulin-Sensitive Fatty Acid Transporter Involved in Diet-Induced Obesity. ***Molecular and Cellular Biology*** 26, 3455-3467.
18. 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-alpha gene with beef fatty acid composition. ***Animal Genetics*** 41, 417-20.