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Analysis of the *PPARGC1A* Gene as a Potential Marker for Productive and Reproductive Traits in Cattle

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An association analysis between T19C polymorphism in bovine *PPARGC1A* and productive and reproductive traits was performed in a population of 620 and 279 Polish Holstein-Friesian cows, respectively. No relationship was found with milk yield and composition. *PPARGC1A* genotypes had, however, a significant effect on lengths of calving interval and calving to conception interval, and the T allele was demonstrated to have an unfavourable effect on these traits. As the identified associations might result from linkage between the T19C and unknown functional polymorphism, further analysis of the *PPARGC1A* is necessary to identify the causative gene variation. The first step in searching for new polymorphism was computer analysis of both promoter and 3'UTR gene sequences which resulted in the prediction of binding sites for several transcriptional factors, including CREB.

Key words: *PPARGC1A* gene polymorphism, reproduction, cattle.

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Peroxisome proliferator-activated receptor gamma coactivator 1 alpha, encoded by the *PPARGC1A* gene (also known as *PGC-1α*), is a multifunctional coregulator of cellular energy metabolism. PPARGC1A was shown to interact with and enhance the activity of many nuclear hormone receptors and transcriptional factors involved in regulation of adaptive thermogenesis, fiber-type switching in skeletal muscle, adipogenesis and gluconeogenesis (for a review see HANDSCHIN & SPIEGELMAN 2006; LIANG & WARD 2006; LIU & LIN 2011). Recently, it was also demonstrated to regulate processes affecting reproduction (TCHEREPANOVA *et al.* 2000; CHEN *et al.* 2008; YAZAWA *et al.* 2010; ZHU *et al.* 2010).

In humans, the *PPARGC1A* gene seems to be involved in the pathogenesis of disorders such as obesity, diabetes, and cardiomyopathy (LIU & LIN 2011). In cattle, it was proposed as a candidate gene for milk-related traits (WEIKARD *et al.* 2005; KHATIB *et al.* 2007; KOMISAREK & DORYNEK 2009; KOWALEWSKA-ŁUCZAK *et al.* 2010), growth and meat quality (SORIA *et al.* 2009), milk fat composition (SCHENNINK *et al.* 2009), as well as functional traits (KOMISAREK & DORYNEK 2009).

Bovine *PPARGC1A* is located in chromosome 6 and consists of 13 exons (WEIKARD *et al.* 2005). Several polymorphisms were identified within this gene (WEIKARD *et al.* 2005, SCHENNINK *et al.* 2009; SORIA *et al.* 2009). Significant associations with phenotypic traits were found mostly for a T/C substitution in intron 9 (known as T19C or c.1892+19T>C) and an A/C polymorphism in the 3'UTR region (A968C or c.3359A>C), although results are inconsistent between studies (WEIKARD *et al.* 2005; KHATIB *et al.* 2007; SCHENNINK *et al.* 2009; KOWALEWSKA-ŁUCZAK *et al.* 2010). In previous research, we found a correlation between T19C and bull breeding value for milk composition and non-return rate in heifers (KOMISAREK & DORYNEK 2009).

Neither T19C nor A968C are located within any consensus sequence for transcriptional factors (KOMISAREK & DORYNEK 2009). As intronic and 3'UTR substitutions, they can, however, alter mRNA splicing or stability. Alternatively, the associations between both SNPs and phenotypic variation might result from linkage with unknown functional polymorphism.

The aim of this study was to confirm the effect of T19C on productive and reproductive traits in dairy cows and to perform a sequence analysis of *PPARGC1A* regulatory regions for the presence of putative transcription factor binding sites as the first step in searching for new polymorphisms.

Material and Methods

All investigations followed the requirements of ethics and were approved by the Local Ethics Commission for Investigation on Animals (permission No. 25/2008).

The association study between T19C polymorphism and traits of interest included 620 Polish Holstein-Friesian cows originating from two herds. In the first one, the mean milk yield per first-lactation cow per 305-day lactation was 7364 kg with 3.95% of fat and 3.33% of protein, whereas the respective values in the second herd were 5976 kg of milk, 3.97% of fat and 3.27% of protein. The animals analysed were daughters of 171 sires, with sire half-sib family sizes varying between 1 and 49.

Phenotypes representing dairy production traits: milk yield (MY, kg), fat yield (FY, kg), fat percentage (FP, %), protein yield (PY, kg) and protein percentage (PP, %) were recorded on a routine basis and expressed as yields of 305-d lactation. Only data from the first lactation of each cow was considered. Phenotypes describing reproductive performance included the number of inseminations per conception in heifers (INCH, inseminations), number of inseminations per conception in first-lactation cows (INCC, inseminations), age at first calving (AFI, days), length of first gestation (GL, days) as well as calving interval (CI, days) and interval from calving to conception (CCI, days) after first calving. Out of 620 animals analysed in this study, the reproductive traits data were available only for 279 cows.

The nucleotide sequence of bovine *PPARGC1A* gene was examined for the presence of putative transcription factor (TF) binding sites with the use of the Genomatix MatInspector software (www.genomatix.de). The analysis was performed for the 2830-bp sequence of the promoter region (GenBank accession no. AY547550S1, position 1-2830) and for the 3843-bp sequence of the 3'UTR region (GenBank accession no. AY321517, position 2482-6324).

DNA for molecular analyses was extracted from peripheral blood using the standard phenol chloroform procedure. Genotypes at T19C *locus* were determined with the PCR-RFLP as described by KOMISAREK & DORYNEK (2009).

Associations between *PPARGC1A* polymorphism and productive and reproductive traits in cattle were analysed with the procedures of the SAS 9.1 package (SAS Institute Inc. 2002-2005). The impact of T19C genotypes was tested by ANOVA, followed by the Duncan test. The statistical model included effects of sire, T19C genotype as well as herd, year and season of calving. Additionally, the allele substitution effects were estimated by regressing the number of copies of the T19C allele C against the analysed trait value.

The chi-square test was used to verify if the allele segregation conformed to the Hardy-Weinberg equilibrium.

Results and Discussion

In the examined group of 620 cows, 37 TT, 268 TC and 315 CC genotypes were identified. The genotypes were distributed according to the Hardy-Weinberg equilibrium. Allele frequencies (T – 0.28 and C – 0.72) obtained in this study did not differ notably from those previously reported for other HF cattle populations (KOMISAREK & DORYNEK 2009; SCHENNINK *et al.* 2009). In Jersey cows, the T allele frequency was shown to be slightly higher – 0.63 (KOWALEWSKA-ŁUCZAK *et al.* 2010).

PPARGC1A plays an important role in lipid metabolism and was proposed as a candidate gene underlying the QTL variation for milk fat-related traits previously described on bovine chromosome 6 (KÜHN *et al.* 1999). WEIKARD *et al.* (2005) reported an association between T19C polymorphism and milk fat yield in a German HF cattle population which, however, was not confirmed in other studies (KHATIB *et al.* 2007; KOMISAREK & DORYNEK 2009; SCHENNINK *et al.* 2009). Also in this research, the *PPARGC1A* effect on milk yield and composition, including fat production, was statistically insignificant (data not shown). However, we found a relationship between the T19C polymorphism and two reproductive traits in cattle (Table 1). Cows with the TT genotype were characterized by a longer calving interval and calving to conception interval than those carrying the other two genotypes. Allele substitution effects only tended to be significant ($P \leq 0.1$), suggesting a partially non-additive mode of inheritance. In a previous study, the T allele was also demonstrated to have an unfavourable effect on reproduction, specifically on bull breeding value for non-return rate in heifers (KOMISAREK & DORYNEK 2009).

There is increasing evidence that *PPARGC1A* can be involved in regulation of physiological processes influencing reproduction. TCHEREPANOVA *et al.* (2000) demonstrated that the product of this gene can associate with estrogen receptor (ER) and may have a role in estrogen signalling. According to CHEN *et al.* (2008), ER interaction with PPARGC1A is important for the FSH and TGFß1-promoted steroidogenesis. Recently, it was shown that PPARGC1A co-activates the steroidogenic factor 1 to influence progesterone production in ovarian granulose cells (YAZAWA *et al.*

Table 1

Least square means of reproductive traits in cows with various T19C genotypes and regression coefficients for the number of copies of the $T19C^C$ allele representing half of the allele substitution effects (± standard errors)

Trait*	Genotype effects				Allele substitution effects	
	TT (n=18)	TC (n=117)	CC (n=144)	Overall P	α/2	P
INCH	1.03±0.22	1.07±0.25	1.15±0.56	0.254	0.08±0.06	0.193
INCC	2.14±1.49	2.16±1.48	2.11±1.39	0.851	-0.33±0.12	0.785
AFI	805.36±64.10	800.69±68.05	801.69±71.00	0.920	-0.54±8.17	0.851
CCI	163.95^{ab}±79.16	137.70^{a}±71.81	129.32^{b}±64.14	0.048	-4.51±7.63	0.091
CI	443.33^{ab}±80.44	417.91^{a}±72.54	407.04^{b}±64.14	0.037	-7.23±7.67	0.083
GL	278.73±6.59	278.45±6.39	277.88±6.43	0.194	-0.53±0.79	0.498

*INCH – insemination number per calving in heifers, INCC – insemination number per calving in cows, AFI – age at first insemination, CCI – calving to conception interval, CI – calving interval, GL – gestation length
a,b – means bearing the same superscript letter differ at $P \leq 0.05$.

2010) and to increase the secretion of LH in pituitary (ZHU *et al.* 2010).

In the last decades, strong genetic progress achieved in milk yield led to continuing decline of cow fertility and the resultant loss of income from dairy production. Identification of genes involved in the decrease of reproductive performance might be useful to improve selection through marker assistance. The results of our study suggest that *PPARGC1A* could be an example of such genes. However, its associations with fertility traits were not tested in other cattle populations. Moreover, T19C was not proved to be the causative polymorphism.

Computer analysis of the *PPARGC1A* gene regulatory regions with the Genomatix MatInspector software resulted in the prediction of several binding sites for transcriptional factors. Results with sequence similarity of 100% are listed in Table 2. One of the positive matches found within the promoter region of *PPARGC1A* was the cAMP-responsive element binding proteins (CREB) consensus sequence. CREB is a well-known transcription factor that regulates pro-

Table 2

Putative binding sites for transcriptional factors within regulatory regions of bovine *PPARGC1A*

PPARGC1A gene region	Transcription factor family	Position*
promoter	EVI1– myleoid transforming protein	237-253
	TALE homeodomain class recognizing TG motifs	1212-1228
	TALE homeodomain class recognizing TG motifs	1256-1272
	cAMP-responsive element binding proteins (CREB)	2693-2713
	Ubiquitous GLI – Krueppel like zinc finger involved in cell cycle regulation	2698-2710
3'UTR	C-abl DNA binding sites	2608-2618
	ZF5 POZ domain zinc finger	2770-2784
	Myeloid zinc finger 1 factors	2832-2842
	TALE homeodomain class recognizing TG motifs	3358-3374
	Myeloid zinc finger 1 factors	3932-3942
	TALE homeodomain class recognizing TG motifs	5270-5286
	Zinc finger transcription factor RU49, zinc finger proliferation 1 – Zipro1	5629-5635

*According to GenBank, accession no. AY547550S1 (promoter) or AY321517 (3'UTR)

liferation and differentiation of various cell types, including neuronal, immune and gonad cells (WEN *et al.* 2010; SCOBEY *et al.* 2001; LONZE & GINTY 2002). Future studies aimed at the identification of *PPARGC1A* functional polymorphism should focus on CREB and other TF consensus sequences found in this research.

In conclusion, the T19C substitution in the bovine *PPARGC1A* gene was not confirmed to influence milk production traits. We found, however, significant associations with lengths of both calving interval and calving to conception interval, suggesting that *PPARGC1A* may be useful for more effective marker-assisted selection to increase the reproductive performance in dairy cattle. T19C is not necessarily the causative polymorphism affecting quantitative trait variability in cattle. To identify the functional genetic variation, future studies will be necessary. Effects of *PPARGC1A* polymorphisms on reproductive traits should also be confirmed in other cattle populations.

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