Associations between the bovine *PPARGC1A* gene and milk production traits

I. Kowalewska-Łuczak, H. Kulig, M. Kmieć

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

ABSTRACT: The aim of this study was to investigate associations between *PPARGC1A* genotypes and haplotypes and milk production traits. The study included 181 Jersey cows. The genotypes were identified by the PCR-RFLP method. The frequencies of the most common alleles were as follows: *T* – 0.63 (c.1892T>C) and *A* – 0.88 (c.3359A>C). The frequency of the most common haplotype was *TC/AA* – 0.558. The results showed that there were no statistically significant associations between the individual genotypes of both SNPs and milk traits; however, individuals with the *CC/AC* haplotype produced significantly ($P \leq 0.05$; $P \leq 0.001$) less milk and had a significantly ($P \leq 0.001$) higher protein content in milk.

Keywords: cattle; *PPARGC1A* gene; PCR-RLP; milk traits

Peroxysome proliferator-activated receptor-γ coactivator-1α (*PPARGC1A* termed PGC-1α) plays a key role in the activation of various nuclear hormone receptors and transcription factors regulating energy balance. It has also been demonstrated that *PPARGC1A* mediates the expression of genes involved in oxidative metabolism, adipogenesis and gluconeogenesis (Puigserver and Spiegelman, 2003). *PPARGC1A* plays a significant role in many aspects of glucose and fat metabolism and energy balance and is able to coordinate the metabolic processes of the liver, fat and muscle tissue in humans and mice. This suggests that *PPARGC1A* might regulate metabolic processes during lactation in dairy cattle and therefore *PPARGC1A* might potentially be the main mediator of the metabolic demands that accompany the onset and progression of lactation in dairy cows (Weikard et al., 2005).

The bovine *PPARGC1A* gene has been mapped to chromosome 6 (BTA6). This gene consists of 13 exons and is expressed at different levels in a great number of tissues (Weikard et al., 2005). Several nucleotide substitutions have been found within the *PPARGC1A* gene, two of which may underlie the variability of milk production traits. One of the substitutions has been mapped to position 1892 (c.1892T>C) in intron 9, and the other has been mapped to position 3359 (c.3359A>C) in the 3'UTR region (GenBank accession No. AY321517). It has been shown that the transition in intron 9 is linked to milk yield and fat yield and content, while the transversion in the 3'UTR region is also associated with milk yield and fat yield and content as well as protein content in milk (Weikard et al., 2005; Khatib et al., 2007).

The aim of this study was to determine gene and allele frequencies at the *PPARGC1A* locus and investigate possible associations between the *PPARGC1A* genotype and combined genotype variants and milk production traits in Jersey cattle.

MATERIAL AND METHODS

The analysis covered 181 Jersey cows kept on a farm located in the Wielkopolska region in Poland. All the animals were kept in identical environmental conditions. They were fed standard feed rations

and seasonally (in spring and summer) put out to pasture. The cows were milked twice a day with the use of a pipeline machine. The herd's milk yield was evaluated by the A4 method in compliance with the recommendations of the International Committee for Animal Recording (ICAR).

DNA was isolated from blood samples collected into test tubes containing K_3EDTA. DNA isolation was performed using a Master Pure kit (Epicenter Technologies, Madison, WI, USA) in accordance with the manufacturer's instructions. Genotype analyses were performed using the PCR-RFLP method.

Two polymorphisms in the *PPARGC1A* gene were analyzed. The polymorphic sites were found to be situated in intron 9 (*T/C* at position 1892) and in the 3' untranslated region (*A/C* at position 3359) of the *PPARGC1A* gene (GenBank accession number AY321517). The primers used to amplify two fragments of the *PPARGC1A* gene had been designed by Khatib et al. (2007). DNA amplification was performed using initial denaturation at 95°C for 5 min followed by 32 cycles of: denaturation at 94°C for 45 s, annealing at 50°C for 45 s and extension at 72°C for 45 s, ending with final extension at 72°C for 7 min (Khatib et al., 2007). The amplified 195 bp (intron 9) and 357 bp long PCR products were digested with *Hae*III and *Nhe*I restriction enzymes, respectively. The restriction fragments obtained were analyzed on a 2.5% agarose gel stained with ethidium bromide. The gels were visualized under UV light and documented with the use of a Vilber Lourmat imaging system.

The analysis of associations between the *PPARGC1A* genotypes and milk production traits – daily milk yield (kg), fat content (%) and protein content (%) – was performed according to the GLM procedure using Statistica Software, version 7.1. (Statsoft, 2006). The following multiple-factor mixed nested model was applied:

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + f_m(a_i) + e_{ijklm}$$

where:

y_{ijklm} = observed value

μ = trait mean

a_i = *PPARGC1A* genotype/combined genotype effec (i = 1, 2, 3, ..., 6)

b_j = lactation number effect (j = 1, 2, 3, 4, 5)

c_k = lactation season effect (k = 1, 2, 3, 4)

d_l = lactation month effect (l = 1, 2, 3, ..., 14)

$f_m(a_i)$ = cow effect, random factor nested within *PPARGC1A* genotype/combined genotype (m = 1, 2, ..., 181)

e_{ijklm} = random error

Differences in the mean values of the traits were tested by Duncan's multiple range test.

RESULTS

The genotype frequencies of both polymorphic sites within the *PPARGC1A* gene in the studied herd of Jersey cows are shown in Table 1. In the case of the SNP site in intron 9, all three genotypes were observed, while in the 3'UTR SNP no homozygous genotype *CC* was found. The frequencies of the most common alleles in the studied herd were as follows: allele *T* 0.63 (c.1892T>C) and allele *A* 0.88 (c.3359A>C). Our study also included an analysis of the *PPARGC1A* gene combined genotypes. The highest frequency was recorded for the *TC/AA* combined genotype (0.558), whereas – due to the lack of the *CC* genotype – the c.3359A>C SNP was found not to contain three combined genotypes: *TT/CC*, *TC/CC* and *CC/CC* (Table 1).

Table 2 shows the effect of the analyzed *PPARGC1A* polymorphisms and combined genotypes on milk production traits in the studied breed.

Table 1. Frequencies of genotypes and combined genotypes of *PPARGC1A* gene

	c.1892T>C			c. 3359A>C		
	TT	*TC*	*CC*	*AA*	*AC*	*CC*
Frequency	0.27	0.72	0.01	0.76	0.24	0
	c.1892T>C/c.3359A>C					
	TT/AA	*TT/AC*	*TC/AA*	*TC/AC*	*CC/AA*	*CC/AC*
	0.199	0.067	0.558	0.166	0.005	0.005

Table 2. Mean and standard deviation (SD) of studied traits in reference to *PPARGC1A* genotype and combined genotype

Genotype	*n*	Milk (kg) ± SD	Fat (%) ± SD	Protein (%) ± SD
c.1892T>C				
TT	357	15.73 ± 4.65	5.80 ± 0.96	4.12 ± 0.55
TC	1 021	15.01 ± 4.69	5.82 ± 1.06	4.10 ± 0.54
CC	17	13.12 ± 5.27	6.58 ± 1.13	4.43 ± 0.80
Total	1 395	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55
c.3359A>C				
AA	1 066	15.04 ± 4.70	5.84 ± 0.10	4.16 ± 1.12
AC	329	15.60 ± 4.68	5.75 ± 1.15	4.04 ± 0.57
Total	1 395	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55
c.1892T>C/c.3359A>C				
TT/AA	261	15.78 ± 4.73^{a}	5.85 ± 0.99	4.18 ± 0.56^{a}
TT/AC	91	15.72 ± 4.51^{b}	5.69 ± 0.85	3.96 ± 0.49^{b}
TC/AA	797	14.81 ± 4.67^{A}	5.84 ± 1.00	4.15 ± 1.25^{c}
TC/AC	229	15.69 ± 4.68^{c}	5.73 ± 1.22	4.06 ± 0.57^{d}
CC/AA	9	14.38 ± 4.98^{B}	6.23 ± 0.62	4.05 ± 0.46^{e}
CC/AC	8	11.70 ± 5.54^{ABabc}	6.98 ± 1.45	4.85 ± 0.92^{abcde}
Total	1 395	15.17 ± 4.70	5.82 ± 1.04	4.11 ± 0.55

$^{A}P \leq 0.05$; $^{a}P \leq 0.001$

n = number of test milk yield; milk (kg) = daily milk yield; fat (%) = fat content; protein (%) = protein content

No differences were found in milk production traits between different c.1892T>C genotypes. It was found, however, that individuals with genotype *CC* were characterized by different average values of the analyzed traits and they had a lower milk yield and a higher fat and protein content in milk than the other cows. There was no significant difference in the analyzed milk production traits between cows with different c.3359A>C genotypes, either. The absence of differences in the analyzed traits in relation to particular genotypes may result from the fact that the c.3359A>C polymorphism did not show the presence of genotype *CC*.

However, the analysis of associations between the *PPARGC1A* gene combined genotypes (c.1892T>C/c.3359A>C) and milk production traits showed some statistically significant ($P \leq 0.05$; $P \leq 0.001$) differences between individual combined genotypes. Individuals with the *CC/AC* combined genotype had a significantly lower milk yield and higher protein content than the other cows.

DISCUSSION

Gene mapping is an essential step in the search for QTLs affecting production traits in livestock. The next step is to identify the gene or allele that leads to the formation of a particular phenotype. In the case of dairy cattle, the greatest emphasis is on the detection of QTLs responsible for milk production and physiological processes that may affect milk production traits such as milk yield, protein and fat yield, and protein and fat content in milk, e.g. genes involved in somatotropic axis (*GH*, *HGR*, *POU1F*) or *DGAT1* gene (Hradecká et al, 2008; Pan et al., 2008).

This study investigated associations between different *PPARGC1A* genotype and combined genotype variants and milk production traits in Jersey cattle. The *PPARGC1A* was chosen due to its crucial role in a wide variety of biological responses including adaptive thermogenesis, mitochondrial biogenesis, and glucose and lipid metabolism

(Liang and Ward, 2006). Another reason for studying the *PPARGC1A* gene was the fact that several QTL studies had shown that this gene is located in the region of bovine chromosome 6 which affects milk production and health traits in dairy cattle (Khatkar et al., 2004).

In this study, the genotype frequencies for the c.1892T>C and c.3359A>C polymorphisms differed a little from the frequencies reported by other researchers. In the case of the c.1892T>C polymorphism, the most frequent genotype was the *TC* genotype, which is a result similar to that obtained by Khatib et al. (2007) (*TC* – 0.65), while Weikard et al. (2005) found the *CC* genotype to be most frequent (0.68). In our study, however, the *CC* genotype had the lowest frequency (0.02), whereas Weikard et al. (2005) and Khatib et al. (2007) reported the lowest frequency for the *TT* genotype (0.02). A comparison of the genotype distribution for the c.3359A>C polymorphism in this study and in works by other authors also showed differences. In this study, the *AA* genotype was the most frequent and the *CC* genotype was not found at all. The frequencies reported by Weikard et al. (2005) and Khatib et al. (2007) were as follows: the highest frequency was found for the *AC* genotype (0.50 and 0.58, respectively) while the lowest frequency was observed for the *AA* genotype (0.16 and 0.15, respectively). The above analysis of data from literature and our own studies shows that it is necessary to do further research as the frequencies of the genotypes presented above are different. This diversity of results could be explained in various ways. One reason might be the variety of the studied breeds (Jersey, Holstein and German Holstein cattle) and the fact that one of the genotypes (*CC*) of the c.3359A>C polymorphism was not found in the present study. Another reason could be a small number of herds of cattle analyzed in relation to literature data.

A statistical analysis of the results obtained in this study showed no statistically significant differences between the genotypes and milk production traits, which is consistent with the results reported by other authors (Weikard et al., 2005; Khatib et al., 2007). Despite the absence of a statistically significant relationship some associations can be noticed between the genotypes and the analyzed traits. Both in our study and in Wikard et al. (2005), cows with the c.1892T>C *TT* genotype produced more milk than other cows. In contrast to Wikard et al. (2005) and our study, Khatib et al. (2007) found a significant association between the c.3359A>C SNP and milk production traits (protein percentage and milk yield). On the other hand, it is worth mentioning that all the studies revealed an association between allele *A* and milk production traits. These discrepancies between the results obtained in this study and those cited from the works of both authors could be explained by different genetic backgrounds of the studied populations and different number of individuals in each population.

It is interesting to note that the statistical analysis done in this study showed statistically significant associations between milk production traits and combined genotypes, which in the case of the c.1892T>C SNP confirms the link between genotypes and the analyzed traits. These results coincide with the results reported by Weikard et al. (2005).

In summary, the results of the statistical analysis of the *PPARGC1A* genotypes and combined genotypes indicate that the *PPARGC1A* polymorphisms can be used in selection for milk production traits. However, further studies are needed to verify the associations between the *PPARGC1A* genotypes and combined genotypes and milk production traits. In addition, the results obtained in our study should be confirmed in larger numbers of cows of different breeds representing all possible genotypes and combined genotypes.

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Corresponding Author

Dr. Inga Kowalewska-Łuczak, Department of Genetics and Animal Breeding,West Pomeranian University of Technology, ul. Doktora Judyma 6, 71 466 Szczecin, Poland
Tel. +48 91 449 6780, e-mail: Inga.Kowalewska-Luczak@zut.edu.pl