ANXA9, SLC27A3, FABP3 and *FABP4* single nucleotide polymorphisms in relation to milk production traits in Jersey cows

H. Kulig, I. Kowalewska-Łuczak, M. Kmieć, K. Wojdak-Maksymiec

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

ABSTRACT: Milk components originating from blood plasma substrates are synthesized in epithelial cells of the mammary gland. Milk lipids are synthesized from fatty acids which bind to specific proteins – FABPs (fatty acid binding proteins). FABPs are a family of small cytoplasmic proteins; nine members of the family have been identified so far (*FABP*1–*FABP*9) (Chmurzyńska et al., 2006). Their main roles include fatty acid uptake, transport and metabolism. FABPs can modulate the fatty acid concentration in cells and therefore they affect different cellular processes, especially lipid metabolism. FABP3 and FABP4 are present in tissues with a high demand for fatty acids, such as heart muscle, skeletal muscles, lactating mammary gland, liver or adipose tissue (Roy et al., 2003). *FABP3* gene was mapped to bovine chromosome 2 (Calvo et al., 2004), where QTLs affecting milk fat yield and content were described (Khatkar et al., 2004). *FABP4* gene was mapped to BTA14 (Michal et al., 2006), which is very rich in QTLs for milk production traits (Khatkar et al., 2004). Fatty acid transport is assisted by the specific proteins called FATPs (fatty acid transport proteins). This protein group includes SLC27A3 (solute carrier family 27, member 3). It belongs to the family of proteins that facilitate long-chain fatty acid transport across the cytoplasmic membrane. Another protein with similar functions is *ANXA9* (annexin A9), the member of Ca^{2+} and phospholipid-binding protein family (Calvo et al., 2006b). Genes encoding *SLC27A3* and *ANXA9* were mapped to chromosome 3, within the region where QTLs for milk fat content and the other milk traits have been mapped. Both of the above-mentioned genes are expressed in the mammary gland (Calvo et al., 2006b). The polymorphic sites (SNPs – single nucleotide polymorphisms) within the bovine *FABP3*, *FABP4*, *SLC27A3* and *ANXA9* genes were identified (Wu et al., 2005; Calvo et al., 2006b; Michal et al., 2006; Cho et al., 2008). Associations between SNPs in these genes and milk production traits in cattle have not been reported so far. However, polymorphism in the *FABP4* gene has been significantly associated with carcass traits in cattle (Michal et al., 2006; Cho et al., 2008). Due to their expression sites, physiological properties and chromosomal localisation, the described genes might be considered as candidate genes for milk production traits. The aim of this study was to determine allele and genotype frequencies and to establish possible associations between the *ANXA9*, *SLC27A3, FABP3* and *FABP4* SNPs, and selected milk traits in Jersey cows.

Keywords: gene polymorphism; dairy cattle; milk traits

MATERIAL AND METHODS

The study included a total of 180 Jersey cows kept on a farm located in the Wielkopolska (Great Poland) region in Poland. All the studied animals came from 17 sires. The animals were kept in identical environmental conditions and were fed a standard diet.

Genomic DNA was extracted from blood using MasterPure™ Genomic DNA Purification Kit (Epicentre® Biotechnologies, Madison, USA) according to the manufacturer's instructions.

The analysed polymorphic sites were as follows:

1. *A*/*G* transition in position 951 of the *ANXA9* gene (GeneBank acc. No. AY785287);
2. *C*/*T* transition in position 2566 of the *SLC27A1* gene (GeneBank acc. No. AY995157);
3. *A*/*G* transition in the *FABP3* gene described by Wu et al. (2005);
4. *G*/*C* transversion in the *FABP4* gene described by Michal et al. (2006).

FABP3 and *FABP4* genotypes were identified according to Wu et al. (2005) and Michal et al. (2006), respectively. In the case of *ANXA9* and *SLC27A3*, primers were designed on the basis of the appropriate GeneBank sequences mentioned above. Afterwards, restriction enzymes for SNP identification were matched by using the NEBcutter (version 2.0) software tool. *Nla*III and *Taq*I restriction endonucleases were chosen for *ANXA9* and *SLC27A3* genotyping, respectively.

The next stage involved a statistical analysis of associations between the SNPs and milk production traits: daily milk yield (kg), fat content (%) and protein content (%), as well as somatic cell count (SCC) in milk. Milk yield was evaluated by the A4 method in compliance with the recommendations of the International Committee for Animal Recording (ICAR). SCC data was recorded on the basis of monthly milking tests. SCC was transformed into the natural logarithm scale (ln SCC). The analysis was carried out using the GLM (General Linear Model) multiple-factor mixed nested model (Statistica, 2006). The following linear model was applied:

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

where:

y_{ijklm} = observed trait value in *ijklm*-th animal
μ = mean trait value for herd
a_i = effect of genotype (i = 1, 2, 3)
b_j = effect of lactation number (j = 1, 2, ..., 5)
c_k = effect of lactation season (k = 1, 2, 3, 4)
d_l = effect of lactation month (l = 1, 2, ..., 14)
$f_m(a_i)$ = effect of cow, random factor nested within genotype (l = 1, 2, ..., 180)
e_{ijklm} = random error

The differences between mean trait values were verified by Duncan's multiple range test.

RESULTS

In the case of *ANXA9* polymorphism, the 241 base pair PCR product digested with *Ssi*I enzyme revealed a non-cutting fragment (allele *G*) and cutting fragments of 202 and 39 bp (allele *A*). The *SLC27A3* PCR product (237 bp) was digested with *Taq*I enzyme into 197 and 41 bp restriction fragments (allele *T*). Allele *C* (non-cutting fragment) was not identified in the studied herd. Moreover, no cows with the *FABP4-GG* genotype were identified. The frequencies of the analysed genotypes and alleles are presented in Table 1.

The study results show statistically significant differences between the mean values of protein and fat content in Jersey cows with different *FABP3* genotypes (Table 2). The *AA* cows were characterised by a significantly ($P \leq 0.01$ and $P \leq 0.05$) higher protein content in milk than the *AG* and *GG* cows. The difference between the *AA* and *AG* individuals averaged

Table 1. Genotype and allele frequencies of the studied SNPs

SNP	Genotype frequencies		Allele frequencies	
ANXA9	*AA*	0.29	*A*	0.56
	AG	0.53		
	GG	0.18	*G*	0.44
FABP3	*AA*	0.58	*A*	0.76
	AG	0.36		
	GG	0.06	*G*	0.24
FABP4	*CC*	0.89	*C*	0.95
	CG	0.11	*G*	0.05

Table 2. Means and standard deviations of milk traits in cows with different *ANXA9* and *FABP3* genotypes (*n* – number of observations)

Genotype	*n*	Daily milk yield (kg)	Fat content (%)	Protein content (%)	*n*	SCC (ln)
ANXA9						
AA	413	14.9 ± 4.6	5.82 ± 1.06	4.12 ± 0.55	707	$5.162798 \pm 1.183045^{A}$
AG	723	15.3 ± 4.6	5.79 ± 0.98	4.10 ± 0.56	1 304	$5.154805 \pm 1.160561^{B}$
GG	259	15.3 ± 5.0	5.93 ± 1.14	4.09 ± 0.54	548	$5.377809 \pm 1.133376^{AB}$
FABP3						
AA	870	15.0 ± 4.6	5.96 ± 1.06^{AB}	4.14 ± 0.54^{Ab}	1 456	5.115622 ± 1.155626
AG	490	15.2 ± 4.8	5.65 ± 0.95^{AC}	4.06 ± 0.56^{A}	900	5.320919 ± 1.124473
GG	88	16.7 ± 4.6	5.50 ± 0.98^{BC}	4.07 ± 0.55^{b}	203	5.329216 ± 1.334948

the means in columns marked with the same superscript letter differ significantly; capital letters denote significance of difference at $P \leq 0.01$, whereas small letters denote significance of difference at $P \leq 0.05$

0.08%; the difference between the *AA* and *GG* cows was slightly lower (0.07%). Similarly, milk fat content in the *AA* cows was significantly ($P \leq 0.01$) higher compared with the *AG* and *GG* cows (by 0.31 and 0.46%, respectively). Moreover, the *AG* cows were characterised by a significantly higher ($P \leq 0.01$) fat content compared with the *GG* cows, the difference averaging 0.15%.

Table 2 shows mean SCC values in cows with different *ANXA9* genotypes. The analysis revealed statistically significant ($P \leq 0.01$) differences between the mean SCC values in Jersey cows with different genotypes. Compared with the *AA* and *AG* genotype cows, ln SCC was lower in the *GG* cows by 0.215011 and 0.223004, respectively. No associations were found between the *FABP4* polymorphism and the traits analysed in this study.

The total variability of fat percentage was explained in 4.2% by *FABP3* polymorphism; 23.6% and 35.6% variability was explained by cow and residual, respectively. In the case of protein percentage, 2.2%, 17.4% and 31.8% variability was explained by *FABP3* genotypes, cow and residual, respectively. In the case of SCC, 2.4%, 23.6% and 62.9% variability was explained by *ANXA9* genotypes, cow and residual, respectively.

DISCUSSION

The candidate gene approach is applied with regard to genes whose products might affect production traits as well as genes neighbouring the known QTLs. Among the most extensively studied genes in dairy cattle are genes encoding milk proteins (e.g. caseins), hormones and its receptors (e.g. growth hormone, leptin) as well as transcriptional factors (e.g. PIT-1). Statistically significant associations between polymorphisms in these genes and milk production traits have been found (Kamiński et al., 2002; Liefers et al., 2002; Zwierzchowski et al., 2002; Maj et al., 2004; Kulig, 2005). Other genes of interest in this area include genes encoding enzymes which participate in fatty acid metabolism as well as genes encoding fatty acid binding and transport proteins (Hradecká et al., 2008).

In the case of the *ANXA9* and *FABP3 loci*, all the genotypes were observed within the studied herd. As regards the *SLC27A3 locus*, the herd proved to be monomorphic. This fact may be explained by breed or population differences, especially as other cattle breeds (Holstein, Pirenaica and Brown Swiss) have been found to be polymorphic (Calvo et al., 2006b). Another reason may be that the studied mutation is so rare that it can be detected only in a sufficiently large population.

Genetic variation at the *ANXA9* and *FABP3 loci* was observed in the studied herd of Jersey cows. In the case of fat percentage, protein percentage and SCC, the estimated variability is in correspondence with the heritability of these traits. The heritability values estimated in Jersey cows ranged from 0.38 to 0.72 for fat percentage and from 0.34 to 0.57 for protein percentage (Roman et al., 2000). In the case of SCC, the heritability was estimated as low and in Jersey cows it ranged from 0.04 to 0.18 (Mostert et al., 2004).

The results obtained in this study demonstrate that there are associations between the *ANXA9* polymorphism and SCC in milk of Jersey cows. The *A* allele is associated with decreased somatic cell count without any decrease in protein content in milk. *ANXA9* and *SLC27A3* are considered as candidate genes for fat content in sheep milk. Calvo et al. (2006a) found no direct associations between the SNPs within these genes and estimated breeding value for milk traits in Manchega breed. However, the quoted authors suggested that these genes are linked to a QTL with some effect on milk traits. *ANXA9* may be thought as candidate gene for milk production traits, as well as for SCC in cattle, with regard to its localisation in the proximity of QTL for these traits (Ogorevc et al., 2009). No association studies concerning the above genes and milk production traits in cattle have been reported so far.

The results of this study also show that the *FABP3* SNP significantly affects fat and protein content in the milk of the studied herd of Jersey cows, with *A* as a desirable allele for improving these traits. It is worth mentioning that cows with this allele have the lowest somatic cell count compared with the other *FABP3* genotype cows, but this difference is not statistically significant. Chromosomal localisation of *FABP3* suggests that it may be considered as candidate gene for milk production traits.

No associations were found between the *FABP4* gene polymorphism and the milk traits analysed in this study. However, this polymorphism was significantly associated with marbling and subcutaneous fat depth in Wagyu × Limousin F2 population (Michal et al., 2006) and in Korean native cattle (Cho et al., 2008).

The presented results suggest that the *FABP3* genotypes might be used to increase fat and protein content in milk and *AA* genotype cows should be preferred in selection for improving these traits. Moreover, preference of cows with the *ANXA9 A* allele might contribute to a decreased SCC in Jersey cattle. These results may be considered for use in selection programmes for milk production traits in Jersey cattle. The heritability of protein and fat content indicates that selection for these traits may be effective. The low heritability of SCC shows that selection for this trait may be difficult, but it is possible. However, further studies are necessary to verify the results of our study. As current knowledge in this field is very limited, research on associations between the *ANXA9*, *SLC27A3*, *FABP3* and *FABP4* genotypes and milk production traits in cattle should be continued.

REFERENCES

Calvo J.H., Marcos S., Jurado J.J., Serrano M. (2004): Association of the heart fatty acid-binding protein (*FABP3*) gene with milk traits in Manchega breed sheep. Animal Genetics, 35, 347–349.

Calvo J.H., Martínez-Royo A., Beattie A.E., Dodds K.G., Marcos-Carcavilla A., Serrano M. (2006a): Fine mapping of genes on sheep chromosome 1 and their association with milk traits. Animal Genetics, 37, 205–210.

Calvo J.H., Martínez-Royo A., Silveri L., Floriot S., Eggen A., Marcos-Carcavilla A., Serrano M. (2006b): Isolation, mapping and identification of SNPs for four genes (*ACP6, CGN*, *ANXA9*, *SLC27A3*) from a bovine QTL region on BTA3. Cytogenetic and Genome Research, 114, 39–43.

Chmurzyńska A. (2006): The multigene family of fatty acid-binding proteins (FABPs): Function, structure and polymorphism. Journal of Applied Genetics, 47, 39–48.

Cho S., Park T.S., Yoon D-H., Cheong H.S., Namgoong S., Park B.L., Lee H.W., Han C.S., Kim E.M., Cheong I-C., Kim H., Shin H.D. (2008): Identification of genetic polymorphisms in *FABP*3 and *FABP*4 and putative association with back fat thickness in Korean native cattle. BMB Reports, 41, 29–34.

Hradecká E., Čítek J., Panicke L., Řehout V., Hanusová L. (2008): The relation of *GH*1, *GHR* and *DGAT*1 polymorphisms with estimated breeding values for milk production traits of German Holstein sires. Czech Journal of Animal Science, 53, 238–245.

Kamiński S., Rymkiewicz-Schymczyk J., Wójcik E., Ruść A. (2002): Associations between bovine milk protein genotype and haplotypes and the breeding value of Polish Black-and-White bulls. Journal of Animal and Feed Science, 11, 205–221.

Khatkar M.S., Thomson P.C., Tammen I., Raadsma H.W. (2004): Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genetics Selection Evolution, 36, 163–190.

Kulig H. (2005): Associations between leptin gene polymorphism and some milk performance traits of cattle. Journal of Animal and Feed Science, 14, 235–243.

Liefers S.C., Te Pas M.F.W., Veerkamp R.F., van der Lende T. (2002): Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in Holstein heifers. Journal of Dairy Science, 85, 1633–1638.

Maj A., Strzałkowska N., Słoniewski K., Krzyżewski J., Oprządek J., Zwierzchowski L. (2004): Single nucleotide polymorphism (SNP) in the 5'-noncoding region of the bovine growth hormone receptor gene and its association with dairy production traits in Polish Black-and-White cattle. Czech Journal of Animal Science, 49, 419–429.

Michal J.J., Zhang Z.W., Gaskins C.T., Jiang Z. (2006): The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu × Limousin F2 crosses. Animal Genetics, 37, 400–402.

Mostert B.E., Banga C., Groeneveld E., Kanfer F.H.J. (2004): Breeding value estimation for somatic cell score in South African dairy cattle. South African Journal of Animal Science, 34, 32–34.

Ogorevc J., Kunej T., Razpet A., Dovc P. (2009): Data base of cattle candidate genes and genetic markers for milk production and mastitis. Animal Genetics, 40, 832–851.

Roman R.M., Wilcox C.J., Martin F.G. (2000): Estimates of repeatability and heritability of productive and reproductive traits in a herd of Jersey cattle. Genetics and Molecular Biology, 23, 113–119.

Roy R., Calvo J.H., Hayes H., Rodellar C., Eggen A. (2003): Fine mapping of the bovine heart fatty acid-binding protein gene (*FABP*3) to *BTA*2*q*45 by fluorescence *in situ* hybridization and radiation hybrid mapping. Animal Genetics, 34, 466–467.

STATISTICA (2006): Statistica, version 7.1. (Data Analysis Software System) Statsoft, Poland.

Wu X-L., MacNeil M.D., De S., Xiao Q-J., Michal J.J., Gaskins C.T., Reeves J.J., Busboom J.R., Wright Jr. R.W., Jiang Z. (2005): Evaluation of candidate gene effects for beef backfat via Bayesian model selection. Genetica, 125, 103–113.

Zwierzchowski L., Krzyżewski J., Strzałkowska N., Siadkowska E., Ryniewicz Z. (2002): Effects of polymorphism of growth hormone (GH), Pit-1, and leptin (*LEP*) genes, cow's age, lactation stage and somatic cell count on milk yield and composition of Polish Black-and-White cows. Animal Science Papers and Reports, 20, 213–227.

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Corresponding Author

Dr. Hanna Kulig, Department of Genetics and Animal Breeding, West Pomeranian University of Technology, Doktora Judyma 6, 71 466 Szczecin, Poland
Tel./fax +48 914 496 780, e-mail: Hanna.Kulig@zut.edu.pl