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SHORT COMUNICATION

ASSOCIATIONS OF DGAT1 POLYMORPHISM WITH MILK CHARACTERISTICS IN SLOVAK DAIRY COWS

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ABSTRACT

DGAT1 encodes diacylglycerol O-acyltransferase (EC 2.3.1.20), a microsomal enzyme that catalyzes the final step of triglyceride synthesis. It was shown that the QTL variation is most likely caused by a nonconservative base substitution in the candidate gene DGAT1 changing lysine to alanine (K232A) in the enzyme diacylglycerol O-acyltransferase (DGAT). In particular, the allele encoding the lysine 232 variant proved to be more efficient with regard to milk fat synthesis. The objective of the present study was to determine the allele frequencies and to verify the effects of the two DGAT variants (K232A polymorphism, A and K alleles) on milk production traits in Slovak dairy cows.

Samples of 196 dairy cows originating from 61 sires were genotyped for DGAT1 K232A polymorphism (A and K alleles) using the PCR-RFLP technique. The frequencies of DGAT1 alleles were 0.88 (A) and 0.12 (K). The performance data were collected during one season in order to minimize this effect. The overall milk yield (MILK), fat yield (FAT_Y), fat content (FAT_C), protein yield (PROT_Y), protein content (PROT_C) and age at first calving (AGE1) were studied. The effect of DGAT1 polymorphism on fat and protein content in milk was confirmed. Further study is needed for explanation of effect of DGAT1 on the age at first calving.

Keywords: genetic marker, cattle, acyl-CoA:diacylglycerol acyltransferase gene (DGAT1), PCR-RFLP

INTRODUCTION

Starting with the first systematic search for QTL in cattle (**Georges *et al.,* 1995**) several genome-wide mapping experiments were conducted in all major dairy breeds to detect QTL with an impact on economically important traits **(Bovenhuis and Schrooten, 2002).** Although these enterprises were successful in determining the approximate chromosomal regions most likely harboring QTL variation, the mapping intervals of the QTL were large **(Zhang *et al.,* 1998**). However, the efficient use of QTL information in selective breeding requires precise mapping within a few centimorgans, or preferably, knowledge about the molecular basis of the QTL variation. The latter was recently achieved for a QTL on chromosome 14 with a pronounced effect on milk fat content. It was shown that the QTL variation is most likely caused by a nonconservative base substitution in the candidate gene *DGAT1* changing lysine to alanine (K232A) in the enzyme diacylglycerol *O*acyltransferase (DGAT) **(Grisart *et al.,* 2002; Winter *et al.,* 2002).** In particular, the allele encoding the lysine 232 variant proved to be more efficient with regard to milk fat synthesis.

Recently, **Cases, S. *et al.* (2001)** showed at least two enzymes catalyze the reaction in which diacylglycerol is covalently joined to long-chain fatty acyl-CoAs to form triglycerides as major constituents of fat, including fat of secreted milk. *DGAT1* encodes one of these enzymes, diacylglycerol *O*-acyltransferase (EC 2.3.1.20), a microsomal enzyme that catalyzes the final step of triglyceride synthesis (**Smith, S. J. *et al.,* 2000**). It became a functional candidate gene for lactation traits after studies indicated that mice lacking both copies of *DGAT1* are completely devoid of milk secretion, most likely because of deficient triglyceride synthesis in the mammary gland (**Smith, S. J. *et al.,* 2000**).

The objective of this study was to verified the associations of the K232A polymorphism in acyl-CoA:diacylglycerol acyltranferase-1 gene (DGAT1) with milk characteristics in Slovak dairy cows.

MATERIAL AND METHODS

Dairy cattle from farm Jasová were used to estimate allele frequencies and gene substitution effects for milk, fat, and protein yield, as well as fat and protein content. The herd consisted of 196 dairy cows originating from 61 sires. The performance data were collected during one season in order to minimize this effect. The overall milk yield (MILK), fat yield (FAT_Y), fat content (FAT_C), protein yield (PROT_Y), protein content (PROT_C) and age at first calving (AGE1) were studied.

PCR-RFLP

A restriction fragment length polymorphism assay was applied to diagnose the K232A substitution in DGAT1. The DNA from collected hairy roots was isolated by the Maxwell 16 Magnetic Particle Processor using a Maxwell purification kit (Promega, USA) for tissue following the manufacturer's instructions. PCR – RFLP and microchip electrophoresis were performed according **Bauer *et al.* (2011)**.

Statistical analysis

The UNIVARIATE, CORR and GLM procedures within the SAS 9.2 software were applied. The outliers were identified using the UNIVARIATE procedures. The analysis was performed using 176 animals. The correlations between studied characteristics were calculated using the CORR procedure. The effect of DGAT1 polymorphism on studied characteristics was determined using the linear models with genotype fitted. As the experiment was conducted within one herd during the one season these effects were eliminated. The effect of DGAT1 allele was calculated for the FAT_C, PROT_C and AGE1.

RESULTS AND DISCUSSION

We present evidence that increased milk fat content in Slovak dairy cows is strongly associated with a lysine at position 232 of the protein encoded by bovine *DGAT1*; an alanine at this position is associated with lowered milk fat
content.

We tested 196 animals of Holstein breed for the K232A polymorphism in *DGAT1* gene by PCR-RFLP genotyping **(Bauer *et al.*, 2011)**. All three posible genotypes were identified The basic statistics of the studied characteristics for the whole dataset are shown in Table 1.

Table 1 The basic statistics of the studied characteristics for the whole dataset.

	Mean	**Std. Dev.**	**Min.**	**Max.**
MILK (kg)	11152.34	2131.30	2676.00	18611.00
FAT_Y (kg)	390.46	77.08	88.31	763.05
FAT_C (%)	3.53	0.466	2.40	5.41
PROT_Y (kg)	343.38	62.80	90.98	571.36
PROT_C (%)	3.09	0.20	2.70	3.70
AGE1 (days)	789.69	91.69	585	1468

The correlations between studied milk characteristics and AGE1 are shown in Table 2. The fat yield (FAT_Y) and proteins yield (PROT_Y) were highly positively correlated with the milk yield (MILK), while the content of these components in milk was negatively correlated with milk yield. The FAT_Y was highly positively correlated with the PROT_Y. Also the content of fat (FAT_C) was positively correlated with protein content (PROT_C). There was no correlation shown between all milk characteristics and the age at first calving (AGE1).

Table 2 The correlations between studied milk characteristics and AGE1.

	FAT_Y	FAT_C	PROT_Y	PROT_C	AGE1
MILK	0.78***	-0.30***	0.94***	-0.32***	0.03
FAT_Y		0.34***	0.83***	-0.004	-0.02
FAT_C			-0.16*	0.50***	-0.08
PROT_Y				0.001	0.03
PROT_C					-0.02

*** P<0.001, *P<0.05

The results (coefficient of determination and P value, LS means and substitution effect of K-allele as regression coefficient) of linear models for different milk characteristics and AGE1 are shown in Table 3.

Table 3 The results of linear models for different milk characteristics and AGE1

	R^2	P value	Genotypes			K-allele effect
			AA (137)	KA(35)	KK (4)	
MILK	0.012	0.3517	-	-	-	-
FAT_Y	0.007	0.5421	-	-	-	-
FAT_C	0.099	0.0001	3.45[a]	3.77[b]	3.99[b]	0.303
PROT_Y	0.001	0.8914	-	-	-	-
PROT_C	0.056	0.0068	3.07[a]	3.17[b]	3.22[a,b]	0.096
AGE1	0.033	0.0533	798.43[a]	760.71[b]	742.00[a,b]	-34.4

[a,b] – LS means with different letters differed significantly

The number of animals with different genotypes are shown in Table 3, the frequencies of DGAT1 alleles were 0.88 (A) and 0.12 (K).

The effect of DGAT1 polymorphism on fat content was determined. The linear model with only the genotype effect significantly explained 10% of overall variability of FAT_C. The similar model for protein content significantly explained 5.6% of overall variability of PROT_C. These results are in agreement with those reported by **Signorelli *et al.* (2009)** who confirmed increased fat and protein content associated with 232K allele of DGAT1 in Holstein and Jersey breed. **Thaller *et al.* (2003)** reported the effect of DGAT1 K232A polymorphism on the all yield and content milk traits. The linear model for the age at first calving explained 3.3% of overall variability of AGE1.

The DGAT1 allele substitution effect (represented as regression coefficient) was determined by regression of number of K-allele copies for the fat content, protein content and age at first calving. The substitution effect of K-allele was 0.303%, 0.096% and -34.4days respectively and was statistically significant. **Thaller *et al.* (2003)** reported the regression coefficients representing the half of the K-allele substitution effects on fat content (0.132 – 0.183%) and protein content (0.026 – 0.057%). **Kaupe *et al.* (2007)** reported higher regression coefficients representing the half of the K-allele substitution effects on fat content (0.29%) and protein content (0.07%). The models including the effect of genotype and the sire explained 50% of overall variability of fat content and 24% of overall variability of protein content. When the

model for joint analysis was applied (including the CYP11B1 polymorphism) the allele substitution effect of DGAT1 did not decreased.

CONCLUSION

We herein generate genetic and functional data that confirm the causality of the DGAT1 K232A mutation on milk production traits in Slovak dairy cows. The effect of DGAT1 polymorphism fat and protein content in milk was confirmed. K allele has been under positive selection supporting its effect on the functionality of DGAT1; and demonstrate that the K232A mutation increases the activity of the enzyme in a way that is in agreement with its effect on phenotype. The benefits of the alternative alleles depend on economic weights given to the different milk production traits in the breeding goal. Further study is needed for explanation of effect of DGAT1 on the age at first calving.

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