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DETECTION OF *DGAT-1* GENE POLYMORPHISM IN HOLSTEIN AND SLOVAK SPOTTED CATTLE BREEDS USING A MICROCHIP ELECTROPHORESIS

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ABSTRACT

The aim of this study was to detect the K232A polymorphism in acyl-CoA:diacylglycerol acyltranferase-1 gene (DGAT1) in the Holstein and Slovak spotted breeds in Slovakia using improved primers and high-throughput microchip electrophoresis (MCE). Samples of 87 animals from the Holstein breed and 42 animals from the Slovak spotted breed were genotyped for *DGAT1 K232A* polymorphism (A and K alleles) using the PCR-RFLP technique. We observed a clear resolution of three different genotypes following fast, automated analysis of PCR products by microchip electrophoresis. The Holstein breed showed the frequencies 0.8621 for the A allele and 0.1379 for the K allele. Most animals were AA homozygotes (frequency 0.7356) or AK heterozygotes (frequency 0.2529). We found only one KK homozygote (0.0115) in the tested sample. The observed frequency of the allele K in the tested herd of Slovak Holstein is lower than reported for this breed in other countries. The genotyping data regarding K232A polymorphism in the Slovak spotted breed showed even lower frequency of the allele K (0.0476) than in the Holstein breed. The Chi-square test found a deviation from the Hardy-Weinberg equilibrium of the *DGAT1 K232A* genotypic frequencies for Holstein and Slovak spotted breeds. This disequilibrium could be related either to the sampling of the genotyped animals or can be a result of selection for the milk production. Our results could be used to guide further association studies between this locus and milk traits in these breeds.

Key words: genetic marker; cattle; acyl-CoA:diacylglycerol acyltransferase gene (*DGAT1*); PCR-RFLP; microchip electrophoresis

INTRODUCTION

A QTL with a major influence on several milk production traits, and particularly on fat content has been identified at the centromeric end of bovine chromosome 14 (Heyen *et al*., 1999; Boichard *et al*., 2003). Fine mapping experiments have found a non-conservative K232A polymorphism inside the *DGAT1* gene, which has been proposed as the causative mutation explaining variation due to the QTL (Grisart *et al*., 2002; Winter *et al*., 2002). *DGAT1* encodes the enzyme acyl-CoA: diacylglycerol-acyltransferase that plays a fundamental role in the metabolism of cellular diacylglycerol in physiological processes, such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation, and lactation, involved in the metabolism of triacylglycerol in higher eukaryotes (Cases *et al*., 1998). Additional studies have demonstrated the effect of K232A mutation (substitution of lysine by alanine at the position 232 of *DGAT1* protein) on the function of the resulting enzyme (Grisart *et al*., 2004). Other studies have shown that the 2 alleles at the K232A polymorphism segregate in several breeds from different countries (Kaupe *et al*., 2004). Nevertheless, the estimated frequency of alleles

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varies greatly according to the population considered, which might reflect different breeding objectives regarding milk composition in different countries and breeds. A significant decrease in protein and milk yields, and increase in fat yield was associated with the lysine substitution (K allele). The alanine variant (A allele) was associated with an increase in protein and milk yields and decrease in fat yield (Spelman *et al*., 2002; Thaller *et al*., 2003; Weller *et al*., 2003).

In spite of having the largest effect on milk production traits, K232A is not the only polymorphism affecting milk production. Variable number of tandem repeat polymorphisms in the promoter region of the *DGAT1* gene also affect milk production traits, probably due to variation at the transcriptional level of *DGAT1* gene (Kühn *et al*., 2004).

Microchip electrophoresis has recently attracted much attention in DNA analysis due to its high efficiency, high throughput, time-saving ability, easy operation, and low consumption of samples and reagents (Zhang *et al*., 2003). Some commercial tools, such as the Agilent 2100 Bioanalyzer, Shimadzu MCE2010, and Hitachi SV1100, has been developed, which has greatly promoted the further application of microchip electrophoresis. In microchip electrophoresis (MCE), nucleic acid fragments are separated at high voltage by capillary electrophoresis in a chip with microfabricated channels with automated detection as well as on-line data evaluation. Therefore, the MCE has emerged as an effective tool for electrophoretic separation of a variety of different DNA inputs and providing a viable alternative to conventional agarose or PAGE slab gel electrophoretic applications (Tay *et al*., 2009).

MATERIALS AND METHODS

DNA extraction

The samples of hairy roots were collected from 87 dairy cows of Holstein breed at the farm Jasova and blood samples were taken from 42 dairy cows of Slovak spotted breed by punction of jugular vein. The DNA from hairy roots and blood was isolated by the Maxwell 16 Magnetic Particle Processor using a Maxwell purification kit (Promega, USA) either for tissue or blood following the manufacturer's instructions.

PCR-RFLP

Genomic DNA was genotyped using a PCR-RFLP assay for the locus responsible for the *DGAT1* K232A substitution. Briefly, PCR reactions were performed at a total volume of 20 µL using 10-50 ng genomic DNA as template, 1X PCR buffer, 2.5 mmol.l^{-1} $MgCl_2$, 0.2 mmol.l^{-1} of each dNTP, 5% DMSO, 0.8 U AmpliTaq Gold DNA polymerase (Applied Biosystems, USA) and 0.4 µmol.l^{-1} of each primer. The PCR profile included an initial denaturation step at 95°C for 5 min, 35 cycles of 94°C (60 s), 63°C (60 s) and a final extension step of 10 min at 72°C. Modified primers for the amplification of 352 bp fragment of bovine DGAT1 gene (sequence acc. No. AY065621.1; dgat1 forward: 5´-catcctcttcctcaagctgttct-3´; dgat1 reverse: 5´-gggcgaagaggaagtagtagaga- 3´) were designed using a Primer3 software (http://frodo.wi.mit.edu/ primer3/input.htm).

Restriction endonuclease *CfrI* (Fermentas, Germany) was used to digest a 352 bp PCR product. The uncut fragment represents the lysine variant, whereas the *CfrI* fragments of 199 and 153 bp represent the alanine variant.

Microchip electrophoresis of DNA fragments

PCR products were analysed by the automated microchip electrophoresis system MCE®-2020 MultiNA (Shimadzu, Japan) with a DNA-500 kit according to the manufacturer's protocol. A SYBR Gold fluorescent dye for DNA staining (Invitrogen, USA) and a a 25 bp DNA Ladder (Invitrogen, USA) was used to determine the size of the PCR products.

Statistical analysis

A PowerMarker v.3.25 computational software (Liu and Muse, 2005) was used to calculate allelic and genotypic frequencies and to estimate the deviation from Hardy-Weinberg equilibrium (HWE) for given locus.

RESULTS AND DISCUSSION

We tested 87 animals of Holstein breed and 42 animals of Slovak spotted breed for the K232A polymorphism in *DGAT1* gene using novel primers for PCR-RFLP genotyping. In several previous genotyping studies, primers for the amplification of 411 bp PCR product have been used (Winter *et al*., 2002; Thaller *et al*., 2003; Lacorte *et al*., 2006). Following the digestion of PCR product by *CfrI*, two fragments of 203 and 208 bp representing the alanine variant were generated. The highly similar size of these two fragments makes it difficult to separate them during the standard agarose gel electrophoresis. Gautier *et al*. (2007) avoided a problematic separation using the TaqMan allelic discrimination technique. Moreover, we found an occasional presence of probably unspecific PCR product of cca 410 bp in our previous experiments what complicated further correct genotyping. Therefore we have designed new primers generating the 352 bp PCR product. The uncut fragment represents the lysine variant (K allele), whereas the *CfrI* fragments of 199 and 153 bp represent the alanine variant (A allele). The digestion products could be effectively analyzed

by fast, automated and high-throughput microchip electrophoresis showing a clear separation of three different genotypes (Figure 1).

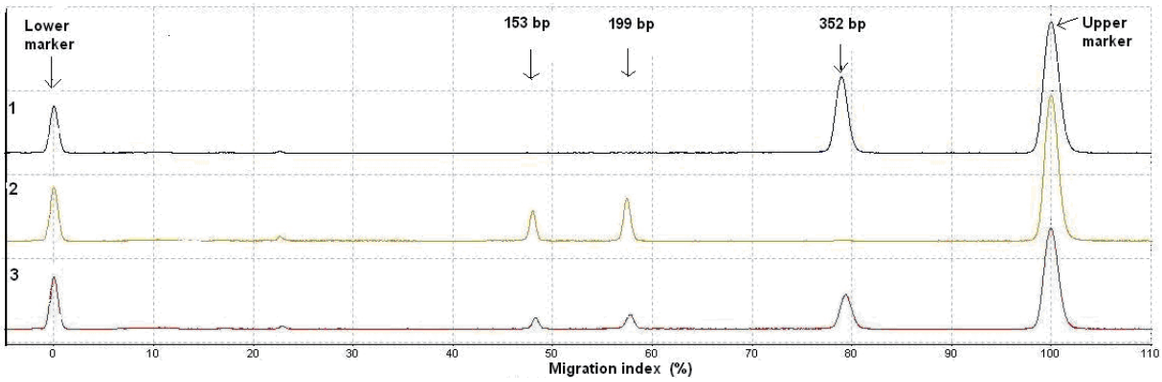

Fig. 1: Representative results of PCR-RFLP genotyping of K232A polymorphism of the *DGAT1* gene in the Holstein breed using the microchip electrophoresis (MCE) Lane 1: genotype KK; Lane 2: genotype AA; Lane 3: genotype KA

Table 1: The allele and genotype frequencies and Hardy-Weinberg equilibrium of *DGAT1* K232A polymorphism in the tested population of Holstein and Slovak spotted breeds

Breed	N	Allele frequency		Genotype frequency			HWE	
		K	A	KK	KA	AA	$\chi 2$	p-value
Holstein	87	0.1379	0.8621	0.0115	0.2528	0.7356	0.3490	0.5547
Slovak Spotted	42	0.0476	0.9524	0	0.0952	0.9048	0.1050	0.7459

The allele and genotype frequencies, as well as the estimation of Hardy-Weinberg equilibrium, were calculated by PowerMarker, v.3.25 and are summarized in Table 1.

The Holstein breed showed the frequencies 0.8621 for the A allele and 0.1379 for the K allele. A majority of tested animals are AA homozygotes (0.7356); we found only one KK homozygote (0.0115). The remaining animals showed the KA genotype (0.2529). The observed frequency of the K allele in the tested herd of Slovak Holstein cattle is lower than reported for this breed in other countries. In German Holstein, allele frequencies reported for the lysine variant K range from 0.35 to 0.548, depending on the sample (Winter *et al*., 2002; Thaller *et al.*, 2003). The French Holstein showed the frequency of the K allele 0.37 (Gautier *et al*., 2007), in the New Zealand 0.30 (Grisart *et al*., 2002), in the Netherland 0.63 (Bovenhuis and Schrooten, 2002) and in the Brazil 0.27 (Lacorte *et al*., 2006).

Our data regarding K232A polymorphism of the *DGAT1* gene in the Slovak spotted breed showed even lower frequency of the K allele (0.0476) than in the Holstein breed. We found 38 AA homozygotes (frequency 0.9048), but only four AK heterozygotes (0.0952) out of 42 tested animals. Such a low frequency was also reported for Fleckvieh cattle (Winter *et al*., 2002; Thaller *et al*., 2003), as well as for Montbeliarde breed (Gautier *et al*., 2007).

The Chi-square test found a deviation from the Hardy-Weinberg equilibrium of the *DGAT1* K232A genotypic frequencies for Holstein and Slovak spotted breeds. This disequilibrium could be related to the sampling of the genotyped animals or can be a result of indirect selection for this locus from the selection for

milk production. In several previous studies a significant decrease in protein and milk yields and increase in fat yield, associated with the lysine substitution (K allele), has been reported. On the contrary, the alanine variant (A allele) was associated with an increase in protein and milk yields and decrease in fat yield (Spelman *et al*., 2002; Thaller *et al.*, 2003; Weller *et al*., 2003).

Our results could be used to guide further association studies between this locus and milk traits, e.g. milk production, fat and protein content in the milk. Well-established diagnostics of *DGAT1* variants in individual animals might be extremely helpful for further investigation of biochemical pathways involved in the expression of milk production traits or for studying possible interactions with other causal genes. In spite of having the strong effect on milk production traits, K232A is not the only polymorphism which affects milk production. Kühn *et al*. (2004) have reported variable number of tandem repeat polymorphisms in the promoter region of the *DGAT1* gene that can also affect milk production traits, probably due to variation at the transcriptional level of the *DGAT1* gene.

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