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Research note

A TaqMan PCR assay for detection of DGAT1 K232A polymorphism in cattle

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Abstract: Metabolic processes involved in the control of fatty acid composition and ratio can greatly influence the quality of cow milk and beef. One of the key elements in this complex pathway is the enzyme diacylglycerol O-acyltransferase 1 (DGAT1). Numerous studies showed a strong correlation between DGAT1 K232A allelic variants and the content of saturated fat in cow milk and the level of beef marbling. PCR-RFLP, the widely used method for SNP detection, is laborious and time-consuming. This article describes the development of an efficient and rapid assay for detection of DGAT1 K232A polymorphism, based on real-time PCR with allele-specific TaqMan probes. The proposed method was validated by PCR-RFLP and the results fully coincided. Thus, our developed assay is efficient and reliable for rapid identification of DGAT1 K and A allelic variants in cattle that can be successfully applied in cattle breeding.

Keywords: DGAT1; cattle; allele; genotype; real-time PCR

1. Introduction

Diacylglycerol O-acyltransferase 1 (DGAT1) (EC 2.3.1.20) is one of the key enzymes involved in triglyceride metabolism. It catalyzes the final stage of triglyceride biosynthesis, using 1,2-diacylglycerol and acyl-CoA as substrates [1]. DGAT1 plays an important role in several physiological processes in higher eukaryotes, such as regulation of triacylgliceride concentration in blood, formation of adipose tissue, maturation of oocytes etc. [2,3]. The deficit of DGAT1 leads to

breakdown of fatty acid synthesis in adipose tissue and skeletal muscles [4], as well as disruption in lactation up to its lack [5].

In cattle (*Bos taurus)* genome the *dgat1* gene is localized in the centromeric region of chromosome 14 together with other genes that determine milk productivity and quality and form the so-called quantitative trait loci (QTL) [6,7]. The study of allelic polymorphism in DGAT1 gene revealed about two dozen single-nucleotide substitutions, most of which were localized in non-coding regions and did not influence the structure of the enzyme [8–11]. Two of the most relevant single-nucleotide polymorphisms (SNP) that alter the amino acid sequence of the DGAT1 protein were found in the coding sequence in exon 8 (rs109234250 and rs109326954): The dinucleotide substitution GC→AA (positions 10433 and 10434 in *dgat1* sequence, GenBank no. AJ318490, position 1802265 and 1802266 on chromosome 14) results in the replacement of alanine with lysine (A→K) at position 232 in the protein. The proposed ancestral K allele was shown to associate with higher activity of the enzyme [8]. The numerous studies conducted on different cattle breeds revealed a link between the presence of the 232K allele and high fat content in cow milk and the predominance of saturated C16:0 and C18:0 fatty acids over unsaturated [8,9,12–14]. There are also data on correlation between K allele and high percent of intramuscular lipid and beef marbling scores [10,15,16]. Thus, *dgat1* is recognized as a candidate gene for the control of fat content and composition in cow milk and beef. The breeding of cattle bearing the A allele can improve the quality of milk by the content of unsaturated fats, while allele K-based selection of animals helps increase the content of saturated fats in milk and meat.

In the current study we aimed to develop a reliable and rapid assay for detection of DGAT1 K232A polymorphism in cattle.

2. Material and methods

2.1. DNA isolation and primer design

The study was conducted using 50 blood samples from cows of Black-and-White holsteinized cattle. Whole blood samples were collected from tail vein with VACUETTE tubes with K3 EDTA (Greiner Bio-One, Austria). The genomic DNA was isolated using the *M-sorb* kit with magnetic beads (Syntol, Russia) according to the protocol provided by the manufacturer.

The *dgat1* gene sequence was retrieved from GenBank (GenBank accession AJ318490). The *GeneRunner* [17] and *Multiple Primer Analyzer* [18] software programs were applied for the design of primers and TaqMan probes.

2.2. Real time-PCR assay conditions and validation

The real-time PCR amplification was run in 10 µL of reaction mix, which contained 5 µL of *LightCycler®480 Probes Master* (Roche, Switzerland), forward primer Dgat1-F: 5'-TGCTGGCCCTGATGGTCTACAC-3' (0.4 µM), reverse primer Dgat1-R: 5'-GCGGTAGGTCAGGTTGTCGG-3' (0.4 µM), and allele-specific fluorescent probes Dgat1-A: 5'-(FAM)-TAAG**GC**GGCCAACGGGGGAGCTGC-(BHQ1)-3' (0.2 µM) and Dgat1-K: 5'-(Cy5)-TAAG**AA**GGCCAACGGGGGAGCTGC-(BHQ2)-3' (0.2 µM). The amount of DNA was 20 ng per reaction.

The real-time PCR was run on *LightCycler® 96* (Roche, Switzerland) under optimized conditions (95 °C for 10 min; 95 °C for 15 sec., 60 °C for 15 sec., 72 °C for 15 sec., 40 cycles). The probe emitted fluorescence was detected at the stage of elongation in FAM and Cy5 channels. The results of genotyping were analyzed using the *LightCycler® 96* thermal cycler software (version SW1.1).

The validation of the developed method was conducted by PCR-RFLP method, described by us earlier [19] (Table 1). Briefly, Dgat1-F: 5'-TGCTGGCCCTGATGGTCTACAC-3' and Dgat1-R2: 5'-GAAGGAAGCAAGCGGACAGT-3' primers were used to amplify 540-bp fragment of *dgat1* gene. PCR was performed with use of qPCRmix-HS kit (Evrogen, Russia). PCR conditions were as follows: 95 °C for 10 min; 95 °C for 15 sec., 62 °C for 15 sec., 72 °C for 15 sec., 40 cycles. The obtained amplicons were digested with 0.2 units of *AcoI* (Sibenzyme, Russia) at 37 °C for 16 h. The restriction products were analyzed by electrophoresis in 1.5% agarose gel [19].

Table 1. Available methods of PCR-RFLP analysis.

Primer sequences and orientation		Restrictase	Genotype profiles (bp)			Reference
			KK	AA	AK	
5'-GCACCATCCTCTTCCTCAAG-3' 5'-GGAAGCGCTTTCGGATG-3'	(+) (−)	CfrI	411	203/208	411/203/208	[8]
5'-TTCCTCAAGCTGTTCTCCTA-3' 5'-CACGTACCTGCTGGATCA-3'	(+) (−)	EaeI	558	369/189	558/369/189	[10]
5'-TGCCGCTTGCTCGTAGCTTTGGCC-3' 5'-ACCTGGAGCTGGGTGAGGAACAGC-3'	(+) (−)	BglI	282/96	254/96/28	282/254/96/28	[20]
5'-TGCTGGCCCTGATGGTCTACAC-3' 5'-GAAGGAAGCAAGCGGACAGT-3'	(+) (−)	AcoI	540	220/320	540/220/320	[19]

3. Results and discussion

To date, PCR-RFLP, direct sequencing, high resolution melting analysis (HRMA) and real-time PCR methods have been proposed for genotyping DGAT1 K 232A polymorphism in cattle [8,10,20–23].

PCR-RFLP is a widely used method for identification of allelic variants of genes. Several PCR-RFLP methods for cattle genotyping for DGAT1 K 232A polymorphism have been proposed [8,10,19,20]. The differences of the available methods concerned the primers used for the specific amplification and the endonuclease applied at the stage of DNA restriction (Table 1). The main advantage of PCR-RFLP is low relative cost of the analysis, but it is a time and labour-consuming (over 12 hours), low productive method. Other important disadvantages of PCR-RFLP include the lack of possibility to automate the analysis and the probability of unreliable results due to non-optimal ratio of the amounts of DNA, restrictase and restriction time. The limiting factor for the application of this method is the absence of appropriate restriction enzymes able to cut the DNA at the desired polymorphic region of the gene [24].

The main disadvantage of direct sequencing is high relative cost for large-scale genotyping. High resolution melting analysis (HRMA) is a simple, rapid, and relatively inexpensive method, but its accuracy depends strongly on the used dye and temperature precision of the instrument [21,25]. Besides, HRMA is sensible to ionic strength of compared samples [26] and is not suitable for genotyping GC-rich regions [27].

The real time-PCR method with allele-specific hydrolysis probes (TaqMan) is an effective alternative to the methods mentioned above. Although real-time PCR (as well as direct sequencing) is more expensive than PCR-RFLP, it has no limitation inherent in HRMA and, in contrast to PCR-RFLP and direct sequencing, can be used for large-scale animal genotyping.

In the current work we developed a method for identification of A and K allelic variants of *Bos taurus* DGAT1 based on real-time PCR technology and TaqMan probes. In the PCR assay we used a set of two primers, which were common for the both *dgat1* alleles, and two allele-specific TaqMan probes. The Dgat1-F and Dgat1-R primers initiate the amplification of 279-bp fragment of the *dgat1* gene. The identification of A and K alleles of DGAT1 is based on the comparison of fluorescence intensity of FAM and Cy5 dyes, respectively. The final values of FAM and Cy5 fluorescence are used to determine the tested animal's genotype. Here we analyzed the genotyping results by the *LightCycler*® 96 thermal cycler software (version SW1.1) that presents the results as a distribution of alleles (Figure 1).

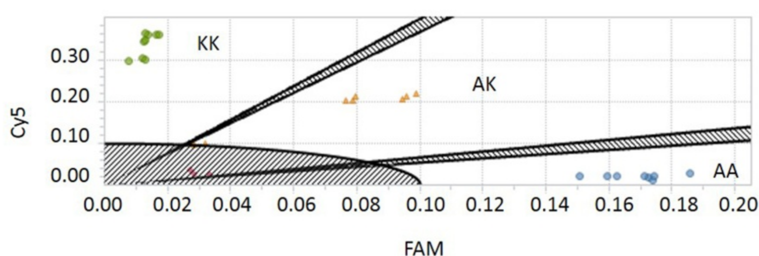

Figure 1. Example of a distribution diagram of DGAT1 A and K alleles.

For samples homozygous for A allele (AA genotype) an increase in fluorescence is detected in FAM channel (Figure 2a). For samples homozygous for K allele (KK genotype) the fluorescent signal is registered in Cy5 channel (Figure 2b). In the case of heterozygous samples (AK genotype) the fluorescent signal is observed by both FAM and Cy5 channels (Figure 2c). Thus, the results of the real-timePCR assay with the allele-specific TaqMan probes allow to unambiguously determine the presence of each of the alleles of DGAT1 gene in the analyzed DNA sample and the animal's genotype, accordingly.

The developed by us TaqMan PCR assay was tested on 50 DNA samples from Black-and-White holsteinized cattle. The results of genotyping showed that 50% of animals carried both DGAT1 alleles (AK genotype), 29.2% were homozygous for A allele (AA genotype) and 20.8% of the tested cows were homozygous for K allele (KK genotype).

In order to validate our method of genotyping DGAT1 K232A polymorphism we conducted PCR-RFLP analysis, described by us earlier [19] (Figure 3). The results of both methods coincided, but despite the low relative cost of PCR-RFLP analysis (the only advantage of the method in comparison with real-time PCR), it is not convenient for genotyping a large number of animals. TaqMan PCR assay is more suitable for large-scale animal genotyping as it helps considerably shorten the time of analysis (up to 1 hour) and avoid operator errors due to one-step procedure.

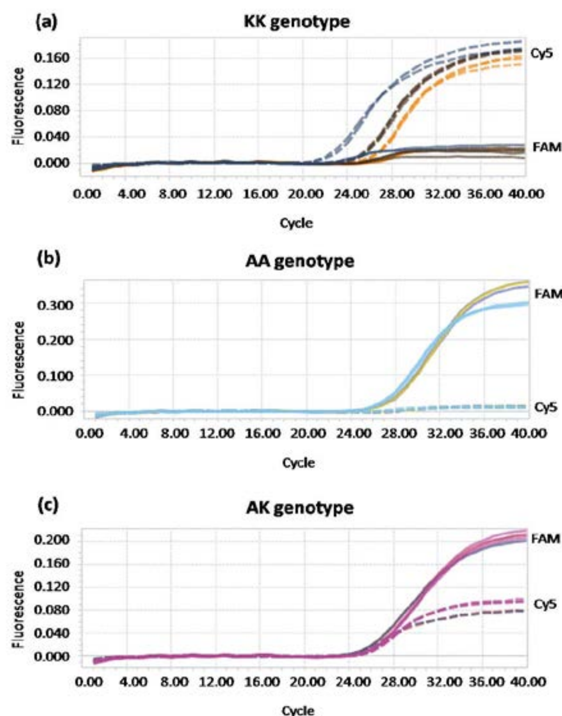

Figure 2. (a) The result of the study of samples homozygous for K allele (KK genotype); (b) the result of the study of samples homozygous for A allele (AA genotype); (c) fluorescence curves for heterozygous samples (AK genotype).

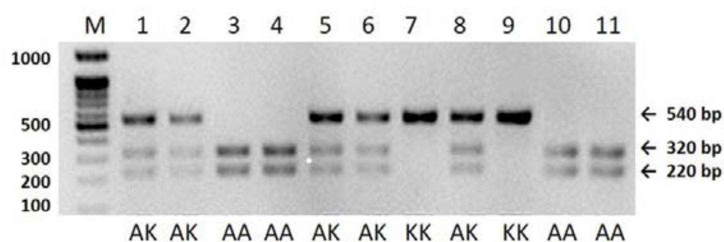

Figure 3. Example of PCR-RFLP genotyping for DGAT1 K232A. The homozygous (AA or KK) and heterozygous (AK) genotypes, along with the corresponding length of the amplified DNA fragments are presented. M—DNA ladder.

Thus, we developed an efficient and reliable method for rapid identification of K and A allelic variants of the *dgat1* gene of cattle based on real-time PCR and using allele-specific fluorescently labelled probes that can be successfully applied in cattle breeding in order to predict the quality of milk and meat from offspring depending on the content of saturated and unsaturated fats.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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