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Analysis of a polymorphism in the DGAT1 gene in 14 cattle breeds through PCR-SSCP methods

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Abstract

The diacylglycerol *O*-acyltransferase (DGAT1) is a microsomal enzyme that catalyzes the final step of triglyceride synthesis. Recent work have evidenced a significant association between lysine at amino acid position 232 with elevated milk fat content, while an alanine at this position is associated with lowered milk fat content. The aim of the present work was to develop a simple and inexpensive PCR-SSCP assay in order to discriminate the CG/AA alleles in exon 8 of the DGAT1 gene. In addition, this method was used to analyze the polymorphism of the DGAT1 through PCR-SSCP methods in 14 populations of cattle from Argentine, Bolivia and Uruguay. The PCR primers were designed from GenBank reported sequences. In this study, we found three PCR-SSCP variants, which were denominated from "A" to "C". However, DNA sequencing analysis showed that "A" variant corresponded with the A allele, while both "B" and "C" observed pattern have the motif AA at positions 10,433–10,434 (K allele), being two alternative conformations of the same DNA sequence. Both variants were detected within each breed with the exception of Hereford, and the heterozygosity varied between 0.000 and 0.524. The gene frequency analysis evidenced significant differences among the studied breeds ($F_{ST} = 0.325$, $p = 0.000$). European *Bos taurus* breeds, with the exception of Jersey breed, showed the lowest frequency of the K allele, while highest K allele frequencies were harboured by *Bos indicus* type cattle. In addition, unselected South American Creole cattle breeds and the synthetic Brangus breed had intermediate allele frequencies.

Keywords: Cattle; Creole breeds; DGAT1; PCR-SSCP

1. Introduction

The presence of a quantitative trait loci (QTL) in the centromeric end of chromosome 14 with a major effects on milk fat content in dairy cattle, has been supported by many studies (Coppieters et al., 1998; Heyen et al., 1999; Riquet et al., 1999; Boichard et al., 2000; Looft et al., 2001). Grisart et al. (2001) reported a strong positional candidate gene for milk fat content in a 3cM interval the diacylglycerol *O*-acyltransferase (DGAT1).

A lysine/alanine (K232A) substitution on the protein encoded by the bovine DGAT1 gene (EC 2.3.1.20) has been shown to be associated with milk fat content in different breeds, such as Holstein-Friesian, Fleckvieh and Jersey (Grisart et al., 2001; Spelman et al., 2002; Winter et al., 2002). In cattle, the lysine variant of DGAT1 is associated with elevated milk fat content, while an alanine at this position is associated with lowered milk fat content. It has been hypothesized that a lysine residue at position 232 of the DGAT1 protein, as it is found in all non-bovine mammalian species studied so far, could confer more efficient binding of acyl-coenzyme A than an alanine residue at this position (Winter et al., 2002), being probably the most likely cause of the BTA14

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QTL effect. Recently, Grisart et al. (2004) provided strong evidence that support the causality of the K232A DGAT1 polymorphism in the determinism of the proximal BTA14 QTL on milk yield and composition. In addition, Thaller et al. (2003) showed that the lysine allele of DGAT1 has also a positive effect on intramuscular fat content in the Charolais and Holstein breeds.

Detection of allelic variation at positions 10,433–10,434 of the DGAT1 gene in different breeds has been performed by diverse assays, such as PCR-RFLP, oligonucleotide ligation assay (OLA), and DNA sequencing (Grisart et al., 2001; Winter et al., 2002; Kaupe et al., 2004). The purpose of the current study was to develop a simple and unexpensive PCR-SSCP assay in order to discriminate the CG/AA alleles in exon 8 of the DGAT1 gene. Such a test could be useful in the genetic characterization of different cattle populations, including studies in the area of population genetics and association analyses with milk production traits.

2. Materials and methods

2.1. Sample collection

Blood samples were collected from 144 unrelated South America Creole cattle from Argentina and Bolivia belonging to the following breeds: Argentine Creole, Saavedreño Creole, Creole of Valle Grande, Chaqueño Boliviano Creole, and Chusco Creole. Also, 118 unrelated animals from European commercial breeds (Hereford, Jersey, Aberdeen Angus, Holstein, Charolais and Normande), 26 bovines from indicine cattle breeds (Nelore and Brahman), and 8 individuals from the synthetic Brangus breed (Br) were sampled.

2.2. DNA extraction and PCR-SSCP DGAT1 analysis

Total DNA was extracted from blood samples using the DNAzol purification kit (Invitrogen, Carlsbad, CA, USA), following manufacturer instructions. PCR primers (F 5′-CTTGCTCGTAGCTTTGGCAGG-3′ and R 5′-CGAAGAGGAAGTAGTAGAGATC-3′) were designed from reported sequences (Genbank accession numbers AY065621 and AJ318490, Grisart et al., 2001 and Winter et al., 2002) in order to amplify a 176 bp fragment of exon 8 of the DGAT1 gene spanning the K232A substitution. The 25 μl reaction mix contained 2 μl of total DNA, 0.4 μM of each primer, 0.1 mM of dNTPs and 0.8 U of *Taq* polymerase (Invitrogen) in 20 mM Tris–HCl (pH 8.4), 50 mM KCl and 4 mM $MgCl_2$, under mineral oil. The PCR consisted in 30 cycles of 45 s at 94 °C, 45 s at 59 °C and 45 s at 72 °C, with a final elongation step of 7 min at 72 °C. Ten microliters of each PCR product was added to 16 μl of loading dye (96% formamide, 0.01 M EDTA pH 8, 0.05% bromophenol blue, 0.05% xylene cyanol) and 10 μl of water. The samples were then heated at 96 °C for 10 min, cooled on ice for at least 5 min and loaded onto a 10% polyacrylamide gel (38:1 acrylamide: bisacrylamide). Electrophoresis was carried out at 4 °C, 3200 V/h in 0.5 × TBE buffer. The gels were subsequently fixed in 5% ethanol, stained with 0.2% $AgNO_3$ and revealed with 2% NaOH. DNA with known genotypes (AA, AK and KK) for the K232A substitution, were use as positive controls.

2.3. DNA sequencing

The PCR products from 8 individuals which presented different PCR-SSCP patterns, including both homozygotes and heterozygotes genotypes were cloned into TOPO TA Cloning kit, according to the manufacturer's instructions (Invitrogen). Several inserts from positive clones for each animal were confirmed by PCR using the primers mentioned above. SSCP patterns were determined in each positive clone and clones corresponding to different PCR-SSCP band patterns were chosen for DNA sequencing. Three clones of each distinct SSCP pattern, that correspond to different clones and individuals, were sequenced in both directions on an Applied Biosystems 377 automated sequencer (Bio-Resource Center, Cornell University, Ithaca, NY, USA), using the T7 universal primer. Sequence was accepted if at least four of six reactions produced identical results at a given base.

2.4. Data analysis

Sequences were aligned using CLUSTAL-W version 1.7 (Baylor College of Medicine, Human Genome Sequencing Center, Houston, TX, USA; Thompson et al., 1994). Gene frequencies were determined for each breed by direct counting. Levels of genetic variability were estimated with the number of alleles (n_a) and the unbiased expected heterozygosity (h_e), computed according to Nei (1987). F_{ST} distances were calculated using the Arlequin software (Schneider et al., 2000). Exclusion power was calculated according to Weir (1996).

3. Results and discussion

Three PCR-SSCP variants were identified, which were at first designated "A" to "C" (Fig. 1). Variants "A" and "B" had the same pattern as control samples corresponding to A and K alleles previously reported by Grisart et al. (2001), respectively. Sequence alignment showed that the "A" and "B" SSCP variants corresponded to haplotypes GC and AA at positions 10,433 and 10,434, respectively. The analysis also evidenced that DNA sequences corresponding to patterns "B"

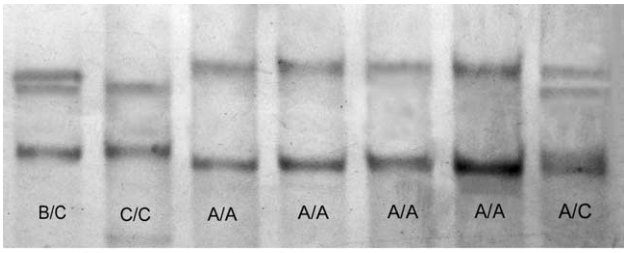

Fig. 1. The DGAT1 PCR-SSCP defined from genomic DNA amplifications in a silver-stained gel. The observed SSCP alleles are indicated at bottom.

and "C" were 100% identical and represented two alternative conformations of the AA haplotype. For this reason, patterns "B" and "C" were designated as variant "B" for allele frequency analysis.

There is solid evidence indicating that DGAT1 underline the QTL on BTA14 affecting milk production traits (Grisart et al., 2004). However, QTL are usually affected by the genetic background and also by environmental factors. So, the effect of the QTL needs validation in different production systems. Our laboratory is especially interested in the characterization of Latin-American native breeds. Centuries of genetic isolation and natural selection for adaptation to tough environments make them a unique genetic resource. Recently, we reported a comparison of allele frequencies of genes related to milk production, growth and immune response in Creole breeds of Argentina and Bolivia (Lirón et al., 2002). We have also conducted association studies between alleles of those genes and milk production in Criollo Saavedreño of Bolivia (Ripoli et al., 2003). Our intention is to include the evaluation of DGAT1 polymorphisms in future experiments involving those breeds. Therefore, we used the newly developed assay to estimate allele frequencies in native breeds, comparing them to breeds of European and indicine origin.

Allele frequencies for all breeds are presented in Table 1. The analysis evidenced highly significant differences among the studied breeds ($F_{ST} = 0.325$, $p < 0.0001$). Both PCR-SSCP variants were detected within each breed, with the exception of Hereford (Table 1).

The allele frequencies of different South American cattle populations showed very consistent patterns. Cebuine breeds had a low frequency of the K allele, in agreement with previously reported results (Winter et al., 2002). Interestingly, European breeds had the K allele at a very high frequency, even those breeds traditionally selected for beef production (Angus, Hereford, Charolais). It is worth noting that the K allele is considered part of the ancestral haplotype of DGAT1 in European populations (Winter et al., 2002). Native Creole breeds tended to have higher frequencies of the K allele, but always below those of European breeds. The fact that native breeds have allele frequencies intermediate to those of European and Cebuine breeds has been confirmed for other genes (Lirón et al., 2002). Little artificial selection has been practiced in native Creole breeds. Since very recently, efforts are being made to create lines adapted to tropical and subtropical environments but with a higher productive potential, as it is the case of the Criollo Saavedreño breed, that is being selected to increase milk production (Wilkins et al., 1983). On the other hand, the introgression of alleles

Table 1
Frequencies of DGAT1 alleles identified by PCR-SSCP analysis

Breed	N	n_a	Gene frequency		h_e	Q^a
			Allele A	Allele B		
European breeds						
Aberdeen Angus	75	2	90.67	9.33	0.170	0.077
Hereford	10	1	100.00	0.00	0.000	0
Holstein	9	2	94.44	5.56	0.111	0.05
Jersey	10	2	65.00	35.00	0.479	0.176
Charolais	10	2	85.00	15.00	0.268	0.111
Average[b]	118	2	89.20	10.80	0.193	0.087
Creole cattle						
Creole Argentine	14	2	57.14	42.86	0.508	0.185
Creole Saavedreño	106	2	63.68	36.32	0.465	0.178
Creole Chaqueño	10	2	65.00	35.00	0.479	0.176
Creole of Valle Grande	11	2	50.00	50.00	0.524	0.187
Average[c]	144	2	63.83	36.17	0.462	0.178
Cebuine breeds						
Nelore	10	2	20.00	80.00	0.337	0.134
Brahman	16	2	21.88	78.13	0.353	0.142
Average	26	2	20.94	79.06	0.331	0.138
Brangus	8	2	62.50	37.50	0.500	0.179

[a] Based on allele frequencies, theoretical exclusion power DGAT1 within breed were calculated according to Weir (1996).
[b] Average value also included four Normande animals.
[c] Average value also included three Creole Chusco animals.

of Cebuine origin in Creole herds has been suspected for a long time and it is a major concern of animal conservationists. In some herds, this suspicion has been recently confirmed by typing specific markers for each subspecies on the Y chromosome (Giovambattista et al., 2000). The allele frequencies of DGAT1 could have been influenced by the introgression process.

There is a growing interest in the detection and characterisation of SNP markers for the purpose of paternity and identity confirmation (Heaton et al., 2002; Werner et al., 2004). The efficacy of these polymorphisms depend of their information content. If the DGAT1 polymorphism is going to be tested in association studies in the population at large, it could be useful to include it in an SNP panel designed for the purposes mentioned above. As expected, estimation of the exclusion power of the DGAT1 marker showed that the information content of this was highly variable, being negligible in some European breeds, moderate in cebuine ones and high in South American Creole cattle and Brangus breeds (Table 1).

In conclusion, the assay based on PCR-SSCP results a cost-efficient method for genotyping of the DGAT1 polymorphism at the population level, providing an alternative to more sophisticated typing methods for small laboratories with limited infrastructure.

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