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SHORT COMMUNICATION

DGAT1 p.K232A polymorphism in dairy and dual purpose Italian cattle breeds

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Abstract

The aim of this study was to evaluate the allele frequency distribution at the *DGAT1* p.K232A polymorphic site in seven Italian dairy and dual purpose cattle breeds. On the whole, 651 animals belonging to Italian Holstein (116), Italian Brown (105), Italian Simmental (95), Valdostana Red Pied (95), Rendena (62), Reggiana (128) and Modenese (50) breeds were genotyped by PCR-RFLP. Sequencing was carried out to confirm results of the genotyping protocol. The *DGAT1* p.232K allele was identified in Italian Holstein (25.4%), Reggiana (17.2%), and with very low frequency in Italian Simmental, Valdostana Red Pied and Rendena (<1%). In Italian Brown and Modenese, this allele was not detected. These results indicated that this polymorphic site can be considered for association studies only in Italian Holstein and Reggiana breeds. Deviation from Hardy-Weinberg equilibrium was observed in the Reggiana breed ($P<0.01$) in which there was an excess of heterozygous sires and absence of animals with the p.232KK genotype. This result should be further evaluated because the analysed sires represented almost all bulls available for artificial insemination in this breed. Comparison of allele frequencies at the *DGAT1* locus with several other Holstein populations showed a wide range of variability, probably due to different selection strategies adopted.

Introduction

In dairy cattle several studies have evidenced the presence of an important QTL in the centromeric region of chromosome 14 (BTA14) affecting mainly milk fat content (fat yield and fat percentage) but also other correlated traits, such as milk yield and milk protein content (protein yield and protein percentage) (Georges *et al*., 1995; Ashwell *et al*., 1998; Bennewitz *et al*., 2004).

Grisart *et a*l. (2002) and Winter *et al.* (2002) have shown that a missense mutation, causing a lysine to alanine substitution at position 232 of the diacylglycerol O-acyltransferase 1 (*DGAT1*) gene product (indicated as p.K232A according to the amino acid change), is the causative mutation of the observed effects. Allele p.232A was associated with higher milk and fat yield and lower fat and protein percentage, compared to allele p.232K. However, other investigations have suggested that additional mutations in this gene or other close genes may be responsible for a portion of the observed effects (Kühn *et a*l., 2004; Fontanesi *et al*., 2005; Gautier *et al*., 2007). These studies were followed by other reports confirming the effects of the p.K232A mutation on milk fat content and composition and other milk production traits, as well as carcass fatness, across a large number of cattle breeds (Spelman *et al*., 2002; Thaller *et al*., 2003a, 2003b; Bennewitz *et al*., 2004; Gautier *et al*., 2007; Schennink *et al*., 2007). Other investigations have indicated association of this mutation with conformational, reproduction, body energy and blood metabolic traits (Kaupe *et al*., 2007; Oikonomou *et al*., 2009).

Here, in order to evaluate the population structures at this important polymorphic site, we analysed the *DGAT1* p.K232A mutation in seven Italian dairy and dual purpose cattle breeds.

Materials and methods

Milk, hair or semen were sampled from a total of 651 animals belonging to seven cattle breeds: Italian Holstein-Friesian, n=116, 43 sires and 73 cows; Italian Brown, n=105 cows; Italian Simmental, n=95 cows; Valdostana Red Pied, n=95 cows; Rendena, n=62 cows; Reggiana, n=128 sires; Modenese, n=50, 21 sires and 29 cows (Table 1). Cows of Italian Holstein Friesian, Italian Brown, Italian Simmental, Valdostana Red Pied, Rendena and Modenese were sampled in several farms in order to increase sire representation. Sire semen was provided by several artificial insemination centres. Almost all active Reggiana sires were analysed.

DNA was extracted from the collected biological materials using the protocols reported in Russo *et al*. (2007). PCR was performed using a PT-100 (MJ Research, Watertown, MA, USA) thermal cycler in a final volume of 20 µL containing the DNA template (about 10-100 ng), 1 U DNA EuroTaq DNA polymerase (EuroClone Ltd., Paington, Devon, UK) 1X PCR buffer, 2.5 mM dNTPs, 10 pmol of each primer (forward: 5'-CCATCCTCTTCCTCAAGCTG-3'; reverse 5'-TAGGTCAGGTTGTCGGGGTA-3') and 2.0 mM of $MgCl_2$. PCR was carried out using the following profile: 5 min at 95°C; 35 amplification cycles of 30 s at 95°C, 30 s at 58°C, 30 s at 72°C; 10 min at 72°C. The amplified fragments of 254 bp (5 µL of PCR product) were digested at 37°C overnight with 5 U of *Cfr*I endonuclease (MBI Fermentas) in a total volume of 25 µL containing 1X reaction buffer. All digested product was electrophoresed in 10% polyacryamide:bisacrylamide 29:1 or 2.0% agarose TBE1X gels. DNA fragments were visualized with ethidium bromide. The genotypes obtained by PCR-RFLP were verified by standard sequencing on both strands from two animals for each genotype. Evaluation of Hardy-Weinberg equilibrium was carried out with χ^2 analysis from the HWE software program (Linkage Utility Programs, Rockefeller University, New York, NY).

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Key words: Allele frequency, *DGAT1*, Italian cattle breeds, QTN.

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Table 1. Genotype and allele frequencies of the *DGAT1* p.K232A polymorphism obtained in the analysed cattle breeds.

		Genotype frequencies (no. of animals)			Allele frequencies	
Breeds	No. of animals	p.232KK	p.232AK	p.232AA	p.232K	p.232A
Italian Holstein	116	0.053 (6)	0.405 (47)	0.542 (63)	0.254	0.746
Italian Brown	105	0.000	0.000	1.000 (105)	0.000	1.000
Italian Simmental	95	0.000	0.011 (1)	0.989 (94)	0.005	0.995
Valdostana Red Pied	95	0.000	0.011 (1)	0.989 (94)	0.005	0.995
Rendena	62	0.000	0.016 (1)	0.984 (61)	0.008	0.992
Reggiana	128	0.000	0.334 (44)	0.656 (84)	0.172	0.828
Modenese	50	0.000	0.000	1.000 (50)	0.000	1.000

Table 2. Allele frequencies of the *DGAT1* p.K232A polymorphism reported in different Holstein populations.

Holstein populations	No. of animals	p.232K	p.232A	Reference
Italy	43 sires + 73 cows	0.25	0.75	This study
Brazil	50 sires	0.27	0.73	Lacorte *et al*., 2006
China	234 cows	0.33	0.67	Xu *et al*., 2006
France	2259 sires	0.37	0.63	Gautier *et al*., 2007
Germany	72*	0.42	0.58	Kaupe *et al*., 2004
Germany	1291 sires	0.55	0.45	Kaupe *et al*., 2007
Germany	833 sires°	0.55	0.45	Thaller *et al*., 2003a
Greece	497 cows	0.62	0.38	Oikonomou *et al*., 2009
Hungary	250 cows	0.84	0.16	Anton *et al*., 2008
Israel	1038 sires#	0.15	0.85	Weller *et al*., 2003
Mexico	196 cows	0.18	0.82	Hori-Oshima and Barreras-Serrano, 2003
New Zealand	1527 sires	0.60	0.40	Spelman *et al*., 2002
Poland	244 sires + 213 cows	0.57	0.43	Pareek *et al*., 2005
Poland	89 sires	0.54	0.46	Nowacka-Woszuk *et al*., 2008
Spain	499 cows§	0.40	0.60	Tupac-Yupanqui *et al*., 2004
The Netherlands	1762 cows	0.40	0.60	Schennink *et al.*, 2007
United Kingdom	571 cows	0.47	0.53	Banos *et al*., 2008

*Sex of the analysed animals was not reported. ° *DGAT1* allele frequencies were estimated based on the maternal alleles of sons in a Granddaughter design. # Estimated allele frequencies. § Daughters of 3 heterozygous sires.

Results and discussion

Allele and genotype frequencies obtained in the seven analysed Italian cattle breeds are reported in Table 1. It is interesting to note that allele p.232K was observed with relatively quite high frequency, compared to the other breeds, only in Italian Holstein (25.4%) and Reggiana (17.2%) breeds. This allele was also detected, but with very low frequency, in Italian Simmental, Valdostana Red Pied and Rendena (<1%). In Italian Brown and Modenese, the p.232K allele was not identified.

Deviation from Hardy-Weinberg equilibrium was observed in the Reggiana breed ($P<0.01$) in which there was an excess of heterozygous sires and absence of animals with the p.232KK genotype. This result was interesting because the analysed sires represented almost all bulls available for artificial insemination in this breed. It could be possible that recent selection activities towards an increase of milk yield in the Reggiana breed, notoriously less productive than the Holstein, might be the causes of the lack of p.232KK sires resulting, as a first step, in a shift towards an excess of heterozygous bulls. This aspect should be monitored and further evaluated as Reggiana milk is usually considered of higher quality due to a higher protein percentage compared to other breeds. A putative change in allele frequencies at the *DGAT1* locus could decrease protein and fat content of the Reggiana milk, worsening rendering capacities.

Other Holstein populations have been investigated for this polymorphism in other studies and comparative allele frequency data are reported in Table 2. A wide range of variability across countries is evident for allele frequencies at this locus. As already observed by Spelman *et al.* (2002), it seems that influences of North American Holstein genetics or selection programs oriented to maximize milk yield, tended to reduce p.232K allele frequency. However, the results reported by Weller *et al*. (2003) showed that allele frequencies of this quantitative trait nucleotide (QTN) could be relatively easily modified by means of variation in selection directions, explaining differences reported in different Holstein populations.

On the contrary, it is interesting to note the absence (or almost complete absence) of variability at the *DGAT1* locus in Italian Brown and Italian Simmental, which confirmed the results reported for the same breeds in other countries (Kaupe *et al.* 2004). In Italian Brown, Bagnato *et al.* (2008) reported the presence of QTL for milk yield and protein percentage in the *DGAT1* region of BTA14, but all sires were homozygous p.232AA (Bagnato *et al*. 2005), as expected by the allele frequencies reported here. This could suggest that other mutations in this locus or in other close genes should be involved in the observed effects, confirming what was reported by Fontanesi *et al*. (2005) in the Italian Holstein population.

Conclusions

This is the first report on *DGAT1* allele frequency distribution in Italian dairy and dual purpose cattle breeds. Variability at the *DGAT1* p.K232A polymorphic site indicated that this locus can be considered for association studies only in Italian Holstein and Reggiana breeds. If the effects of the *DGAT1* mutation will be confirmed in these two populations, as it might be expected from the results reported in other breeds, it could be interesting to evaluate the possibilities to use this QTN for marker assisted selection (MAS). MAS would be particularly interesting in the Reggiana breed, in which it could be difficult to implement genomic selection programs, due to the small population size of this local breed.

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