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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 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 al. (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 al., 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, reproductive, 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 EuroTag DNA polymerase (EuroClone Ltd., Paimington, Devon, UK) 1X PCR buffer, 2.5 mM dNTPs, 10 pmol of each primer (forward: 5’-CCATCCTCTTCCCTCAGCTG-3’; reverse: 5’-TAGTGACATTGCTGGGGA-3’) and 2.0 mM of MgCl₂. 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 endonuclease (MBI Fermentas) in a total volume of 25 μL containing IX reaction buffer. All digested product was electrophoresed in 10% polyacrylamide: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 χ² analysis from the HWE software program (Linkage Utility Programs, Rockefeller University, New York, NY).

**Introduction**

In dairy cattle several studies have evidenced the presence of an important QTL in
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.

Deviations from Hardy-Weinberg equilibrium were 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

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

| Breeds             | No. of animals | Genotype frequencies (no. of animals) | Allele frequencies |
|-------------------|----------------|----------------------------------------|--------------------|
|                   |                | 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 |       | Topac-Yupanqui et al., 2004 |
| The Netherlands      | 1762 cows      | 0.40 0.60 |       | Schenink 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.
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.

References

Anton, I., Kovács, K., Fésüs, L., Várhegyi, J., Lehel, L., Hajda, Z., Polgár, J.P., Szabó, F., Zsolnai, A., 2008. Effect of DGAT1 and TG gene polymorphisms on intramuscular fat and on milk production traits in different cattle breeds in Hungary. Acta. Vet. Hung. 56:181-186.

Ashwell, M.S., Da, Y., Van Tassel, C.P., Van-Raden, P.M., Miller, R.H., Resroad, C.E., 1998. Detection of putative loci affecting milk production and composition, health and type traits in a United States Holstein population. J. Dairy Sci. 81:3309-3314.

Bagnato, A., Schiavini, F., Dolezal, M., Dubini, S., Rossoni, A., Maltecca, C., Santus, E., Medugorac, I., Solkner, J., Fontanesi, L., Friedman, A., Lipkin, E., Soller, M., 2005. The BoVMAS Consortium: identification of QTL for milk yield and milk protein percent on chromosome 14 in the Brown Swiss breed. Ital. J. Anim. Sci. 4(Suppl. 2): 13-15.

Bagnato, A., Schiavini, F., Rossoni, A., Maltecca, C., Dolezal, M., Medugorac, I., Solkner, J., Russo, V., Fontanesi, L., Friedman, A., Soller, M., Lipkin, E., 2008. Quantitative trait loci affecting milk yield and protein percentage in a three-country Brown Swiss population. J. Dairy Sci. 91: 767-783.

Banos, G., Woolliams, J.A., Woodward, B.W., Forbes, A.B., Coffey, M.P., 2008. Impact of single nucleotide polymorphisms in leptin, leptin receptor, growth hormone receptor, and diacylglycerol acyltransferase (DGAT1) gene loci on milk production, feed, and body energy traits of UK dairy cows. J. Dairy Sci. 91:3190-3200.

Bennenwitz, J., Reinsch, N., Paul, S., Loof, C., Kaupe, B., Weimann, C., Erhardt, G., Thaller, G., Kuhn, C., Schwerin, M., Thomsen, H., Reinhardt, F., Reents, R., Kahl, E., 2004. The DGAT1 K232A mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. J. Dairy Sci. 87: 431-442.

Fontanesi, L., Scotti, E., Pecorari, D., Zambonelli, P., Bigi, D., Dall’Olio, S., Davoli, R., Lipkin, E., Soller, M., Russo, V., 2005. The BoVMAS Consortium: Investigation of bovine chromosome 14 for quantitative trait loci affecting milk production and quality traits in the Italian Holstein-Friesian breed. Ital. J. Anim. Sci. 4(Suppl. 2):16-18.

Gautier, M., Capitan, A., Fritz, S., Eggen, A., Boichard, D., Druet, T., 2007. Characterization of the DGAT1 K232A and variable number of tandem repeat polymorphisms in French dairy cattle. J. Dairy Sci. 90: 2980-2988.

Georges, M., Nielsen, D., Mackinnon, M., Mishra, A., Okimoto, R., Pasquino, A.T., Sargeant, L.S., Sorensen, A., Steele, M.R., Zhao, X., Womack, J.E., Hoeschele, I., 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 139:907-920.

Grisart, B., Coppieters, W., Farnir, F., Karim, L., Ford, C., Berzi, P., Cambisano, N., Mni, M., Reid, S., Simon, P., Spelman, R., Georges, M., Snell, R., 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Res. 12:222-231.

Hori-Oshima, S., Barreras-Serrano, A., 2003. Relationships between DGAT1 and Pit-1 genes polymorphism and milk yield in Holstein cattle. J. Anim. Sci. 81(Suppl. 1): 252.

Kaupe, B., Brandt, H., Prinzenberg, E.-M., Erhardt, G., 2007. Joint analysis of the influence of CYP11B1 and DGAT1 genetic variation on milk production, somatic cell score, conformation, reproduction, and productive lifespan in German Holstein cattle. J. Anim. Sci. 85:11-21.

Kaupe, B., Winter, A., Fries, R., Erhardt, G., 2004. DGAT1 polymorphism in Bos indicus and Bos taurus cattle breeds. J. Dairy Res. 71:182-187.

Kühn, C., Thaller, G., Winter, A., Bininda-Emonds, O.R., Kaupe, B., Erhardt, G., Bennenwitz, J., Schwerin, M., Fries, R., 2004. Evidence for multiple alleles at the DGAT1 locus better explains a quantitative trait locus with major effect on milk fat content in cattle. Genetics 167:1873-1881.

Lacorte, G.A., Machado, M.A., Martinez, M.L., Campos, A.L., Maciel, R.P., Verneque, R.S., Teodoro, R.L., Peixoto, M.G.C.D., Carvalho, M.R.S., Fonseca, C.G., 2006. DGAT1 K232A polymorphism in Brazilian cattle breeds. Genet. Mol. Res. 5:475-482.

Nowacka-Woszuk, J., Noskowski, A., Strabel, T., Jankowski, T., Śwіtofіski M., 2008. An effect of the DGAT1 gene polymorphism on breeding value of Polish Holstein-Friesian sires. Anim. Sci. Pap. Rep. 26:17-23.

Oikonomou, G., Angelopoulou, K., Arsenos, G., Zygoyiannis, D., Banos, G., 2009. The effects of polymorphisms in the DGAT1, leptin and growth hormone receptor gene loci on body energy, blood metabolic and reproductive traits of Holstein cows. Anim. Genet. 40:10-17.

Pareek, C.S., Czarnik, U., Zabolewicz, T., Pareek, R.S., Walawski, K., 2005. DGAT1 K232A quantitative trait nucleotide polymorphism in Polish Black-and-White cattle. J. Appl. Genet. 46: 85-87.

Russo, V., Fontanesi, L., Scotti, E., Tazzoli, M., Dall’Olio, S., Davoli, R., 2007. Analysis of melanocortin 1 receptor (MC1R) gene polymorphisms in some cattle breeds: their usefulness and application for breed traceability and authentication of Parmigiano Reggiano cheese. Ital. J. Anim. Sci. 6:257-272.

Schennink, A., Stoop, W., Visker, M.H.P.W., Heck, J.M.L., Bouwens, H., van der Poel, J.J., van Valenberg, H.J.F., van Arendonk, J.A.M., 2007. DGAT1 underlies large genet-
ic variation in milk-fat composition of dairy cows. Anim. Genet. 38:467-473.
Spelman, R.J., Ford, C.A., McElhinney, P., Gregory, G.C., Snell, R.G., 2002. Characterization of the DGAT1 gene in the New Zealand dairy population. J. Dairy Sci. 85: 3514-3517.
Thaller, G., Krämer, W., Winter, A., Kaupe, B., Erhardt, G., Fries, R., 2003a. Effects of DGAT1 variants on milk production traits in German cattle breeds. J Anim Sci. 81:1911-1918.
Thaller, G., Kühn, C., Winter, A., Ewald, G., Bellmann, O., Wegner, J., Zuhlke, H., Fries, R., 2003b. DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. Anim. Genet. 34:354-357.
Tupac-Yupanqui, I., Baro, J.A., Dunner, S., 2004. Effects of DGAT1 alleles on milk and components traits in Spanish Holstein breed. Arch. Zootec. 53:293-299.
Weller, J.I., Golik, M., Seroussi, E., Ezra, E., Ron, M., 2003. Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population. J. Dairy Sci. 86:2219-2227.
Winter, A., Krämer, W., Werner, F.A.O., Kollers, S., Kata, S., Durstewitz, G., Buitkamp, J., Womack, J.E., Thaller, G., Fries, R., 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA: diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. P. Natl. Acad. Sci. USA 99:9300-9305.
Xu, X.R., Gao, X., Xu, S.Z., Zhang, Y.H., Li, J.Y., Ren, H.Y., 2006. Effects of the K232A Substitution at DGAT1 gene on some economic traits in 3 Chinese dairy cattle. J. North. Sci.-Tech. Univ. Agric. Forest. 34:6-10.