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# The *DGAT1* gene *K232A* mutation is associated with milk fat content, milk yield and milk somatic cell count in cattle (Short Communication)

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## Abstract

The present study investigated the *K232A* mutation of the diacylglycerol O-acyltransferase 1 gene (*DGAT1*) in 315 Czech Holstein Cows. The allele frequency was found to be 0.19 for the *K* allele and 0.81 for the *A* allele. The results of *K232A* testing were assessed in relation to average daily milk yield (l), percentage of fat, protein, lactose and milk somatic cell count (SCC, thousand/ml). A GLM procedure was used to analyse the differences among genotypes. The *K232A* genotypes were significantly associated with milk fat percentage (*KK*, *KA*>*AA* [$P \leq 0.005$, $P \leq 0.05$]) and milk yield (*KK, KA*>*AA* [$P \leq 0.05$, $P \leq 0.005$]). The *K* allele was also favourable for SCC levels: cows with the *KA* genotype had lower SCC levels than those with the *AA* genotype ($\boldsymbol{P} \leq 0.05$), while cows with the *KK* genotype showed the lowest levels of SCC at all. This new association of *K232A* suggests the existence of another gene in the centromeric region on BTA14 linked to *DGAT1* with direct effect on the SCC. On the basis of a broad range of *DGAT1* protein functions and the non-conservative matter of *K232A*, a direct effect of *K232A* on the SCC cannot be ruled out either.

**Keywords:** Czech Holstein Cattle, *DGAT1*, *K232A*, milk, SCC

## Zusammenfassung

**Die *DGAT1*-Gen-*K232A*-Mutation hängt mit Milchfettgehalt, der Milchleistung und der somatischen Milchzellzahl beim Rind zusammen** (Kurzmitteilung)

Die *K232A*-Mutation des Diacylglycerin-O-acyltransferase 1-Gens (*DGAT1*) wurde bei 315 tschechischen Holstein-Kühen untersucht. Die relative Häufigkeit des *K*-Allels betrug 0,19, die des *A*-Allels 0,81. Die Ergebnisse der *K232A*-Genotypisierung wurden in Relation zur durchschnittlichen täglichen Milchleistung (l), Fett-, Protein- und Lactosegehalt sowie Anzahl somatischer Zellen (SCC, tausend/ml) bewertet. Zur Auswertung der Unterschiede zwischen den Genotypen wurde ein GLM-Verfahren verwendet. Die *K232A*-Genotypen zeigten einen signifikanten Zusammenhang mit dem Prozentsatz an Milchfett (*KK, KA*>*AA* [$P \leq 0{,}005$, $P \leq 0{,}05$]) und der Milchleistung (*KK, KA*>*AA* [$P \leq 0{,}05$, $P \leq 0{,}05$]). Das *K*-Allel war auch für die SCC-Niveaus günstig: Kühe mit dem *KA*-Genotyp zeigten geringere SCC-Niveaus als diejenigen mit dem *AA*-Genotyp ($P \leq 0.05$), während Kühe mit dem *KK*-Genotyp die geringsten SCC-Niveaus überhaupt zeigten. Dieser neue Zusammenhang von *K232A* legt

die Existenz eines weiteren Gens in der zentromerischen Region auf BTA14 in Verknüpfung mit *DGAT1* mit direkter Auswirkung auf SCC nahe. Auf der Grundlage eines breiten Bereichs der *DGAT1*-Proteinfunktionen und der nicht konservativen Bedeutung von *K232A* kann eine direkte Wirkung von *K232A* auf das SCC-Niveau ebenfalls nicht ausgeschlossen werden.

**Schlüsselwörter:** Holstein Rind, *DGAT1*, *K232A*, Milch, SCC

## Introduction

Quantitative trait loci (QTL) and genes with influence on milk production traits have been the objective of various mapping studies in the last decade. The diacylglycerol O-acyltransferase 1 gene (*DGAT1*) became a candidate gene for milk production traits in dairy cows after experiments showing reduced or inhibited milk secretion in *DGAT1* knock-out mouse lines (Smith *et al.* 2000). After identifying a QTL in the centromeric region of the BTA14 affecting milk fat yield and content (Grisart *et al.* 2002), the *DGAT1* harbouring a non-conservative lysine to alanine substitution (*K232A*) with profound effect on milk fat content became a strong candidate gene for this QTL. Using the positional cloning approach and analysis of the expression of both alleles separately in virus expression systems, the functional and genetic causality of *K232A* to QTL for fat content on BTA14 was confirmed (Grisart *et al.* 2004). Functionally, the *DGAT1* gene is one of at least two enzymes catalysing the final step in triglyceride synthesis in eukaryotic cells (Yen *et al.* 2008). The aim of the present study was to elucidate the effect of *K232A* on selected milk production traits in Czech Holstein Cattle and thus acquire new information about this genetic marker.

## Material and methods

*Animals*

315 Holstein cows which had reached normalised lactation were genotyped for *K232A*. The animals came from one South Moravian breed and had the following composition according to pedigree patterns: HA – 51 % (purebred animals); HB, HC, HD – 49 % (individuals with 50-88 % proportion of purebred pedigree). The tested animals were fed on the same feeding ration. To prevent mastitis, a standard program of hygienic safeguards was carried out in the breed. Milk production trait data were collected in the year 2008. The fat, protein and lactose content were measured automatically using a Bentley 2000 instrument (Bentley Instruments Inc., Chaska, USA). The somatic cell count (SCC) was assessed with a SomaCount 500 instrument (Bentley Instruments Inc., Chaska, USA).

*PCR-RFLP analysis*

The DNA template was isolated from cattle milk somatic cells and blood using the columned method (Jetquick blood and cell culture DNA spin kit, Genomed, St. Louis, MO, USA). PCR was carried out according to Winter *et al.* (2002). The resulting 411 bp PCR product of the *DGAT1* gene was digested overnight using *Cfr*I (3U/5 µl PCR product, New England BioLabs Inc., Ipswich, MA, USA). The results of analysis were visualized on 3.5 % agarose gel by ethidium bromide staining.

*Statistical analysis*

The relative allele and genotype frequencies were estimated for the *K232A* mutation (Liu & Muse 2005). The Hardy-Weinberg equilibrium was tested using the chi-square ($\chi^2$) test. The results of SNPs testing were evaluated in relation to average daily milk yield (l), percentage of fat, protein, lactose and SCC (thousand/ml). The SCC levels were log transformed to meet model assumptions. Observations of a SCC>300 000 cells/ml were not included in the statistical analysis to avoid milk with the characteristics of mastitis. The results of analyses were processed using the Statistica 7 programme (StatSoft 2008). The following GLM procedure was applied to analyse the differences among genotypes:

$$x_{ijk} = m + a_j + b_k + a_j \times b_k + e_{ijk} \quad (1)$$

where $x_{ijk}$ is the value of screened milk performance parameter *i*, with genotype *j*, number of lactation *k*, *m* is the mean value of screened milk performance parameter, $a_j$ is the fixed effect of the genotype *j*, $b_k$ is the fixed effect for the number of lactation *k*, $a_j \times b_k$ is the effect of the interaction between genotype *j* and number of lactation *k*, and $e_{ijk}$ is the residual error.

## Results and discussion

The relative genotypic and allelic frequencies for the *DGAT1* loci are presented in Table 1. The results showed that Hardy-Weinberg equilibrium was not maintained ($P \leq 0.01$). Kuehn *et al.* (2007) reported an analogous frequency of the *K* allele in German Holstein (0.24) and Näslund *et al.* (2008) in Swedish Holstein cows (0.12), the frequency recorded by Hori-Oshima *et al.* (2003) in Mexican Holstein was lower (0.08). Thaller *et al.* (2003), Bennewitz *et al.* (2004), detected markedly higher frequency of the *K* allele in German and Polish Holstein (0.55 and 0.53 respectively). The tested individuals represented a selected, artificial group of non-panmictic animals and the observed Hardy-Weinberg disequilibrium was therefore expected. The impact of inbreeding is unlikely as the animals were chosen from several unrelated sire families.

Table 1
Effect of the *K232A* on milk production traits in Czech Holstein Cattle

| *K232A* | *KK* | | *KA* | | *AA* | | *K* | *A* |
|---|---|---|---|---|---|---|---|---|
| (N=315) | (N=4) | | (N=113) | | (N=198) | | allele freq. | |
| genotype freq. | 0.01 | | 0.36 | | 0.63 | | 0.19 | 0.81 |
| | LSM | SD | LSM | SD | LSM | SD | *P* | |
| Milk yield*, l | **39.17**$^{b}$ | 3.60 | **29.12**$^{A}$ | 7.02 | **20.01**$^{Ab}$ | 7.52 | 0.000 | |
| Fat content, % | **4.55**$^{B}$ | 0.12 | **4.33**$^{a}$ | 0.32 | **3.85**$^{aB}$ | 0.44 | 0.000 | |
| Protein content, % | 3.14 | 0.32 | 3.37 | 0.36 | 3.36 | 0.44 | 0.688 | |
| Lactose content, % | 5.18 | 0.12 | 4.84 | 0.42 | 4.80 | 0.49 | 0.505 | |
| Log SB, thous./ml | 1.47 | 0.45 | **1.94**$^{a}$ | 0.53 | **2.18**$^{a}$ | 0.59 | 0.038 | |

N: number of animals, LSM: least square mean, SD: standard deviation, *average daily milk yield, $^{a,b}P \leq 0.05$, $^{A,B}P \leq 0.005$

The statistical analysis results are presented in Table 1. The *K* allele (lysine variant) was clearly positively associated with milk fat content (*KK*>*AA*, *KA*>*AA*, $P \leq 0.05$). This is in agreement with observations in Holstein breeds presented by other authors (Spelman *et al.* 2002, Weller *et al.* 2003, Thaller *et al.* 2003, Pareek *et al.* 2005, Hradecká *et al.* 2008, Näslund *et al.* 2008). Recent studies have shown that the QTL for fat content on BTA14 is determinated by more polymorphic elements than just the *K232A* (Kühn *et al.* 2004, Gautier *et al.* 2007). The evidence for this are the results of experiments which analysed the haplotypes originating in SNPs in the *DGAT1* locus and other linked genes (Kaupe *et al.* 2007) or haplotypes originating in VNTRs in the *DGAT1* promoter region and *K232A* (Sanders *et al.* 2006). Here, the haplotypes enabled interpretation of a wider genetic basis for the mentioned QTL on BTA14 than the *K232A* alone. Further, Kuehn *et al.* (2007) found different effects of maternal and paternal inherited haplotypes composed of *K232A* and VNTR alleles of the *DGAT1* and from this they inferred the existence of nonadditive interactions in demonstration of *DGAT1* polymorphism.

The *K* allele tended to be favourable for milk yield in the present study as well (*KK*>*AA*, $P \leq 0.05$, *KA*>*AA*, $P \leq 0.005$). Analogous findings were reported by Anton *et al.* (2008) in Hungarian Holstein as well as in Mexican Holstein (Hori-Oshima *et al.* 2003). The highly significant effect of *K232A* in our study was an increase of up to 9,11 l milk yield per day on average in cows with the *KA* genotype compared to those with the *AA* genotype. In contrast, Spelman *et al.* (2002), Thaller *et al.* (2003), Näslund *et al.* (2008), found a positive effect of the *A* allele on total milk yield.

Somatic cell count (SCC) is a key milk quality parameter and mammary gland health status indicator with high economic impact. Using accurate markers in marker assisted selection schemes with the aim of decreasing SCC levels, combined with traditional breeding schemes, could be a useful strategy (Schrotten *et al.* 2004, Kühn *et al.* 2008, Sender *et al.* 2008). Our positive finding is the favourable *K* allele association with lower SCC levels in lactating mammary gland. This association was clear, as the SCC levels decreased linearly according to *K232A* genotypes in the order *AA*>*KA*>*KK*. However, there were significant differences only between the *KA* and *AA* genotypes in SCC values ($P \leq 0.05$). This was most probably due to the low count of cows with the *KK* genotype and this may have affected the statistics. The existence of positive phenotypic and genetic correlations between milk yield and SCC (Samoré *et al.* 2003) do not explain our observation, as the *KK* cows with the lowest SCC also had the highest milk production. The presence of a QTL affecting the SCC in the centromeric region on BTA14 is known (Zhang *et al.* 1998). Sanders *et al.* (2006) found a significant association between specific VNTR allele in the *DGAT1* promoter region and SCC level in the German Angel breed but the effect of *K232A* on the SCC did not reach statistical significance. One possible explanation for the association between *K232A* and SCC levels observed in our study could be the existence of another gene with direct effect on the SCC linked to *DGAT1*. Another explanation could emerge from the specific functions of the DGAT1 protein and its lower activity, when the alanine variant of the *K232A* is present. In general, this has been defined for the ability of the DGAT1 to bind acyl-CoA in triglyceride synthesis (Grisart *et al.* 2004). Significantly higher DGAT protein activity with the *K232A KK* genotype was reported by Sorensen *et al.* (2006) as well by testing *M. longissimus dorsi* microsomal samples from bulls. Triglycerides are not only a basic component of cell energy metabolism; they are also a main constituent of the cell membranes. Hence, the effect of the DGAT1

polymorphism in processes like formation of cell membrane components and cytogenesis of different cell types including as well the genesis of immunocompetent cells could be detectable. As DGAT1 enzymes underlie the esterification of FA (fatty acid) chains, they can also prevent inflammation caused by intracellular FA excess or by FA metabolites toxicity. In a study on transgenic mouse lines with overexpression of *DGAT1*, fed a high fat content diet, phosphorylation of factor JNK1 fundamentally regulating the inflammatory process in skeletal muscle occurred. This suggested that the higher activity of DGAT1 reduces the start of inflammation in skeletal muscle tissue (Liu *et al.* 2007). Compared with the functionally related DGAT2, the DGAT1 protein has additional acyltransferase activity. It catalyses the synthesis of retinyl esters and is also one of the two keys enzymes regulating retinol (vitamin A) synthesis. The retinoids (biologically active retinol forms) generally affect processes like proliferation, cell differentiation, apoptosis, homeostasis and organism immunity. On these grounds, it is hypothesised that polymorphism of *DGAT1* may manifest in the health status of the cow mammary gland eventually.

Compared to analyzing indirect phenotypes like breeding values, analysis of direct phenotype is more suitable for precise estimation of the allele effects at causal mutations. In our study, animals with *K232A* heterozygote genotypes possessed phenotypes differing insignificantly from the midpoint between the phenotypes of the two homozygous genotypes for the analysed milk production traits. This could refer to the presence of a codominance effect, in agreement with Čítek *et al.* (2007), Hradecká *et al.* (2008) and Näslund *et al.* (2008).

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