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Short communication

DGAT1 K232A quantitative trait nucleotide polymorphism in Polish Black-and-White cattle

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Abstract. The diacylglycerol o-acyltransferase 1 gene (*DGAT1*) was investigated in Polish Black-and-White cattle. The frequency of the K allele was 0.60, 0.68 and 0.48 for AI sires (n = 150), young bulls (n = 139) and cows (n = 213), respectively. The method of selective genotyping for identification of the quantitative trait nucleotide was verified through identification of *DGAT1* effect on milk production traits. Daughters of six heterozygous bulls were selectively genotyped based on their milk traits. The genotypic frequencies differed between high and low yield groups representing milk and fat contents. The Kruskal-Wallis test revealed a highly significant effect of *DGAT1 K232A* in cows with extremely low fat content and a significant effect in cows with extremely high protein content of milk. No significant effect of AI sires' genotypes on their breeding value was found.

Key words: *DGAT1*, quantitative trait nucleotide, milk production traits.

Triglyceride synthesis is catalysed by acyl CoA diacylglycerol acyltransferase (DGAT, EC 2.3.1.20). This enzyme plays a fundamental role in the metabolism of cellular diacylglycerol and is important in higher eukaryotes for physiologic processes involving triacylglycerol metabolism, such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation, and lactation (Cases et al. 1998). After the first systematic genome-wide search for quantitative trait loci (QTLs) affecting milk production traits in cattle (Georges et al. 1995), a QTL for fat content was found in the bovine chromosome 14 (Grisart et al. 2002). A construction of a BAC contig spanning the corresponding marker interval of 3 cM revealed a strong candidate gene *DGAT1* harbouring a non-conservative lysine to alanine substitution (*K232A*) with a strong effect on milk fat content and other milk characteristics in the New Zealand, Dutch, German and Israeli Holstein-Friesian (HF) cattle (Grisart et al. 2002; Spelman et al. 2002; Thaller et al. 2003; Weller et al. 2003).

In this paper we investigated the frequency of *DGAT1* (*K232A*) genotypes and its effect on milk production traits in Polish Black-and-White (PBW) cattle using the method of selective genotyping. Selective genotyping was used for two reasons. Firstly, to test and reconfirm the effect of *DGAT1 K232A* quantitative trait nucleotide (*QTN*) polymorphism on milk production traits in PBW cattle (Grisart et al. 2004). Secondly, to verify the selective genotyping experimental design, which is ultimately being utilized on our current genome scan project to identify the multiple QTL regions in PBW cattle using selective DNA pooling approach (Pareek et al. 2002a, 2002b).

105 AI sires, 139 young tested bulls and 213 cows of the PBW breed were genotyped to

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evaluate the distribution of *DGAT1 K232A* polymorphism. To validate the selective genotyping method, the effect of *DGAT1 K232A* variants on five milk production traits-milk yield, fat yield, fat content, protein yield and protein content was investigated in six half-sib families of PBW. In each family, cows representing extremely high and low values (i.e. both tails) of each phenotypic trait were selected from 31 herds. Five percent of best and worse performing cows for each trait were genotyped in each herd. The differences between two means of extreme tails were close to twice the SD values for a trait.

DNA was extracted from the semen and blood using commercially available MasterPure™ DNA Purification Kit (Epicentre, USA). The PCR was carried out in a 10 µl volume containing 15 pmol of each primer set and 5 µL of PCR premix and 0.5 U of *Tfl* DNA polymerase (As supplied by Epicentre, USA) and 50 ng of genomic DNA. A touch down DNA amplification protocol (Don et al. 1991) was applied to obtain the 411-bp gene product with an initial denaturation at 94°C for 4 min followed by 1 min denaturation at 94°C, 1 min annealing at 70°C to a touch down at 60°C and 1 min extension at 72°C, which ended by 30 cycles and a final elongation step at 72°C for 10 min, with the use of a T3 thermocycler (Biometra, Germany). The RFLP assay for *DGAT1 K232A* was performed according to Winter et al. (2002). The 411-bp digested product was visualized on 3% agarose gel (F-MultiImager, Bio-Rad, CA, USA), representing an uncut fragment for the $DGAT1^{K}$ allele (AAG) and two fragments of 203 bp and 208 bp each for the $DGAT1^{A}$ allele (GCG).

The chi-square test was used to verify the similarity of genotypic distributions in best and worse performing cows. The effect of cows' genotypes on each of their five milk traits was tested with the non-parametric Kruskal-Wallis test. The same test was used to verify the effect of sire's genotype on its breeding value.

The genotypic and allelic frequencies are presented in Table 1. Within AI sires the K allele exists in a lower frequency than in young bulls. In cows the frequencies of two variants were almost equal. Thus the status of the PBW population may be characterized as intermediate ($DGAT1^{A+K}$). This intermediate status is also observed in American Holstein cattle (Kaupe et al. 2002). The PBW breed is traditionally upgraded up to 90% with American Holstein blood. Some other cattle breeds, like Jersey, show high frequencies of $DGAT1^{K}$. In German Holstein and German Black Pied cattle, however, the A allele is almost fixed (Thaller et al. 2003).

Table 1. Genotypic and allelic frequency at the *DGAT1 K232A QTN* polymorphic site in Polish Black-and-White cattle

Group	Genotypic frequency			Allelic frequency	
	KK	KA	AA	K	A
AI sires, n = 105	0.32	0.55	0.13	0.60	0.40
Young bulls, n = 139	0.43	0.50	0.07	0.68	0.32
Cows, n = 213	0.19	0.59	0.22	0.48	0.52

Table 2. Genotypic and allelic frequency at the *DGAT1 K232A QTN* polymorphic site in selectively genotyped Polish Black-and-White cows with extreme values of milk production traits

Trait	Genotypic frequency			Allelic frequency	
	KK	KA	AA	K	A
Milk yield					
+ tail, n = 127	0.32	0.56	0.12	0.60	0.40
– tail, n = 113	0.21	0.61	0.18	0.52	0.48
Fat yield					
+ tail, n = 124	0.22	0.63	0.15	0.53	0.47
– tail, n = 117	0.24	0.60	0.16	0.54	0.46
Fat content*					
+ tail, n = 113	0.15	0.60	0.25	0.45	0.55
– tail, n = 120	0.43	0.53	0.04	0.70	0.30
Protein yield					
+ tail, n = 116	0.22	0.61	0.17	0.53	0.47
– tail, n = 113	0.29	0.61	0.10	0.60	0.40
Protein content*					
+ tail, n = 116	0.10	0.62	0.28	0.41	0.59
– tail, n = 108	0.39	0.47	0.14	0.63	0.37

* Frequencies of genotypes differ highly significantly between tails

The distribution of *DGAT1 K232A* genotypes was further analysed in selectively genotyped cows from six half-sib families (here, all bulls are of KA genotypes) (Table 2). The results show a highly significant effect of *DGAT1 K232A* genotypes on fat content and protein content. This is in accordance with the previously observed effects in the New Zealand and Dutch HF (Spelman et al. 2002), Israeli Holstein (Weller et al. 2003) and German Holstein (Thaller et al. 2003).

Based on available breeding values for all five milk performance traits in 107 AI sires, no significant differences between sires' genotypes were

observed. By contrast, Weller et al. (2003) found significant effects of the *K232A* substitution on all milk production traits in the examined Israeli Holstein bulls. The reason for this disparity might be either a lower precision of breeding value estimation or fewer AI sires included in our study.

We also investigated the effect of *DGAT1 K232A* polymorphism in selectively genotyped PBW cows. The non-parametric Kruskal-Wallis test revealed a highly significant effect of *DGAT1 K232A* in cows with extremely low fat content and a significant effect in cows with extremely high protein content of milk.

The results of this study validated the experimental design based on selective genotyping. The identification of *DGAT1* effect in any cattle population not only serves as a good marker to identify the QTL regions for milk production traits but also verifies the experimental design in the bovine genome scan projects using selective DNA pooling methodology. Similarly, Fisher and Spelman (2004) verified the selective DNA pooling methodology through identification and estimation of the *DGAT1 QTN* polymorphism in a New Zealand HF population. The obtained results can be further extended and applied to test the linkage disequilibrium within adjacent haplotype markers (Weller et al. 2003), and to verify other sources of genetic variance in this chromosomal region. Recent studies showed that the *DGAT1 K232A* locus is represented by more than one QTL region (Bennewitz et al. 2004) and multiple QTL alleles at BTA14 affecting milk fat content in cattle (Kühn et al. 2004).

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Key words: DGAT1, quantitative trait nucleotide, milk production traits. Triglyceride synthesis is catalysed by acyl CoA diacylglycerol acyltransferase (DGAT, EC 2.3.1.20). This enzyme plays a fundamental role in the metabolism of cellular diacylglycerol and is important in higher eukaryotes for physiologic processes involving triacylglycerol metabolism, such as intestinal fat absorption, lipoprotein assembly, adipose tissue formation, and lactation (Cases et al. 1998). After the first systematic ge-nome-wide search for quantitative trait loci (QTLs) affecting milk production traits in cattle...Â Table 1. Genotypic and allelic frequency at the DGAT1 K232A QTN polymorphic site in Polish Black-and-White cattle. Group. The diacylglycerol o-acyltransferase 1 gene (DGAT1) was investigated in Polish Black-and-White cattle. The frequency of the K allele was 0.60, 0.68 and 0.48 for AI sires (n=150), young bulls (n=139) and cows (n=213), respectively. The method of selective genotyping for identification of the quantita â€¦Â The method of selective genotyping for identification of the quantitative trait nucleotide was verified through identification of DGAT1 effect on milk production traits. Daughters of six heterozygous bulls were selectively genotyped based on their milk traits. The genotypic frequencies differed between high and low yield groups representing milk and fat contents. In another study in Polish Black-and-White bulls they detected frequencies of 0.38 and 0.62 for K and A alleles, respectively, with excess of KA heterozygotes mainly at the expense of homozygotes AA which according the authors may indicate the presence of overdominant selection (Komisarek and Michalak 2008).Â 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. Komisarek J, Michalak A, Walendowska A 2011: The effects of polymorphisms in DGAT1, GH and GHR genes on reproduction and production traits in Jersey cows. Anim Sci Pap Rep 29: 29-36.