DOI: 10.1534/genetics.103.022749

# Evidence for Multiple Alleles at the *DGAT1* Locus Better Explains a Quantitative Trait Locus With Major Effect on Milk Fat Content in Cattle

**Christa Kühn,* Georg Thaller,† Andreas Winter,† Olaf R. P. Bininda-Emonds,† Bernhard Kaupe,‡ Georg Erhardt,‡ Jörn Bennewitz,§ Manfred Schwerin* and Ruedi Fries†,[1]**

**Forschungsbereich Molekularbiologie, Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, 18196 Dummerstorf, Germany, †Lehrstuhl für Tierzucht der Technischen Universität München, 85350 Freising, Germany, ‡Institut für Tierzucht und Haustiergenetik der Justus-Liebig-Universität, 35390 Giessen, Germany and §Institut für Tierzucht und Tierhaltung der Christian-Albrechts-Universität zu Kiel, 24098 Kiel, Germany*

Manuscript received October 1, 2003
Accepted for publication May 6, 2004

ABSTRACT

A quantitative trait locus (QTL) for milk fat percentage has been mapped consistently to the centromeric region of bovine chromosome 14 (BTA14). Two independent studies have identified the nonconservative mutation *K232A* in the acylCoA-diacylglycerol-acyltransferase 1 (*DGAT1*) gene as likely to be causal for the observed variation. Here we provide evidence for additional genetic variability at the same QTL that is associated with milk fat percentage variation within the German Holstein population. Namely, we show that alleles of the *DGAT1* promoter derived from the variable number of tandem repeat (VNTR) polymorphism are associated with milk fat content in animals homozygous for the allele *232A* at *DGAT1*. Our results present another example for more than two trait-associated alleles being involved in a major gene effect on a quantitative trait. The segregation of multiple alleles affecting milk production traits at the QTL on BTA14 has to be considered whenever marker-assisted selection programs are implemented in dairy cattle. Due to the presence of a potential transcription factor binding site in the 18mer element of the VNTR, the variation in the number of tandem repeats of the 18mer element might be causal for the variability in the transcription level of the *DGAT1* gene.

WITH the development of refined analytical tools in genome analysis, the identification of causal genes and their sequence variation underlying complex traits has become feasible in humans, animals, and plants (Glazier *et al.* 2002). Exact knowledge of the genetic background of complex traits will provide a better understanding of genetic mechanisms and physiological functional pathways and is also a prerequisite of efficient marker/gene-assisted selection schemes. Several studies in cattle described a quantitative trait locus (QTL) with impact on milk production traits, and on milk fat percentage in particular, in the centromeric region of bovine chromosome 14 (BTA14; Riquet *et al.* 1999; Looft *et al.* 2001). Fine-mapping efforts localized the QTL to the marker interval *BULGE09–ILSTS039*, which spans ~3 cM (Farnir *et al.* 2002). A comparative positional candidate gene approach led to the identification of the candidate gene acylCoA-diacylglycerol-acyltransferase 1 (*DGAT1*). *DGAT1* is considered to be the key enzyme in controlling the synthesis rate of triglycerides in adipocytes (Coleman and Bell 1976). Smith *et al.* (2000) described that lactation is absent in knockout mice lacking both copies of *DGAT1*. Mutation analysis revealed 19 polymorphic sites within the bovine *DGAT1* gene (Winter *et al.* 2002). While 17 mutations were located in introns, in the promoter, or in the untranslated exon regions, the GC → AA exchange at positions 10433/10434 (in this article all positions are given according to GenBank no. AJ318490) results in a nonconservative substitution of amino acid 232 (A → K) in the *DGAT1* protein. Thus, this mutation was a strong candidate as the causal mutation underlying the QTL for milk fat percentage in the centromeric region of BTA14. Two independent studies showed a strong association of the *DGAT1 K232A* alleles with milk fat content variation in the Holstein, Jersey, and Fleckvieh breeds, with allele *232K* increasing milk fat content compared to allele *232A* (Grisart *et al.* 2002; Winter *et al.* 2002).

However, these and further studies (Spelman *et al.* 2002; Thaller *et al.* 2003) conducted to estimate the effects of *DGAT1 K232A* showed that effect sizes within families as well as across populations differed to some extent. Apart from the effects of different genetic backgrounds, the question was raised as to whether further sources of genetic variation underlying variability of milk fat percentage might exist in the genomic region of *DGAT1*. While several reports support a diallelic QTL in the centromeric region of BTA14 (*e.g.*, Spelman *et al.* 2002; Boichard *et al.* 2003), Winter *et al.* (2002) put up the

[1]*Corresponding author:* Lehrstuhl für Tierzucht, Alte Akademie 12, 85354 Freising, Germany. E-mail: ruedi.fries@tierzucht.tum.de

hypothesis of multiple QTL alleles. Subsequent support for this hypothesis arose from a sire that is highly probably segregating at the QTL (Thomsen *et al.* 2001), but was shown to be homozygous *AA* for *K232A* (Thaller *et al.* 2003).

Thus, the objective of our study was to formally test the hypothesis of multiple QTL alleles for milk fat content by further investigating promising polymorphisms in *DGAT1*. Apart from the surmises stated above, there are good reasons to expect more than two causative alleles *a priori*. Glazier *et al.* (2002) critically reviewed experiments in which genes influencing complex characters were positionally cloned and reported multiple causal mutations within genes in most cases. Further examples of multiple causal alleles were detected in the bovine myostatin gene responsible for muscular hypertrophy (Dunner *et al.* 2003), in the porcine *PRKAG3* gene affecting glycogen content in the muscle (Ciobanu *et al.* 2001), and in the human *NOD2* gene involved in susceptibility to Crohn's disease (Ogura *et al.* 2001).

Within the *DGAT1* gene, we hypothesized that mutations in regulatory regions were strong candidates for any additional causative variations for three reasons. First, Yu *et al.* (2002) showed that the regulation of *DGAT1* expression in adipocytes occurs largely at the transcriptional and post-transcriptional level. Second, in humans, the *DGAT1* promoter allele *79T* was found to be associated with a reduced promoter activity in cultured adipocytes and a reduced body mass index in a clinical study of 476 females (Ludwig *et al.* 2002). Finally, in cattle, two polymorphisms detected in the chromosomal region 5′ to the transcription start differed strongly in their allelic frequencies when we compared pools consisting of bulls with extreme opposite breeding values for milk fat content: (1) a variable number of tandem repeat (VNTR) polymorphism at position 1465 and (2) a C → T/G mutation at position 3343 (Winter *et al.* 2002). We focused mainly on the VNTR polymorphism in the *DGAT1* promoter because it contains the CCCGCC motif, which is a potential binding site of the transcription factor SP1, and it could be hypothesized that the variation in the number of repetitions of this putatively regulatory element might be causal for the variation of *DGAT1* expression. In addition, the highly probable segregating sire mentioned previously was shown to be homozygous for all polymorphisms detected in *DGAT1* except for the VNTR polymorphism by sequence analysis (our unpublished data).

In this article, we provide evidence that there is indeed further genetic variation for milk fat content in addition to the *DGAT1 K232A* substitution in the targeted chromosomal region. At least part of this variation was shown to be associated with the VNTR polymorphism in the promoter region of the *DGAT1* gene, which may have a functional relevance for *DGAT1* transcription.

## MATERIALS AND METHODS

**Animals:** The pedigree material was collected as a collaborative QTL research effort of German artificial insemination and breeding organizations, scientific institutes for animal breeding, and animal computing centers initiated by the German Cattle Breeders Federation (ADR) and the German Holstein Association (DHV). The data set consisted of 34 paternal half-sib families from the German Holstein breed with a total of 1764 bulls (Table 1). The average family size was 51.9 sons (7–127 sons per family). The basic structure of the pedigree material was a granddaughter design (Weller *et al.* 1990).

**Markers:** For QTL mapping, four markers in the centromeric region of BTA14 were included. Two microsatellite markers (*CSSM066*, *ILSTS039*) were amplified using primer pairs from a public domain database (http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl). Microsatellite genotypes were determined by automated fragment analysis (A.L.F., Amersham, Freiburg, Germany; ABI377, Applied Biosystems, Darmstadt, Germany; Kühn *et al.* 2003). The *DGAT1 K232A* mutation was genotyped according to Winter *et al.* (2002). PCR primers ($DGAT1_{UP}$ 5′-GCA CCATCCTCTTCCTCAAG-3′; $DGAT1_{DN}$ 5′-GGAAGCGCTTT CGGATG-3′) amplified a 411-bp fragment that was digested by the restriction enzyme *Cfr*I (MBI Fermentas, St. Leon-Rot, Germany). The resulting fragments (allele *232K*, one uncut fragment of 411 bp; allele *232A*, two fragments of 203 and 208 bp) were separated on a 2% agarose gel. A PCR fragment containing the VNTR polymorphism at position 1465 in the promoter of the bovine *DGAT1* gene was amplified by the following primers: $DGAT1_{proUP}$ 5′-TCAGGATCCAGAGGTAC CAG-3′ and $DGAT1_{proDN}$ 5′-GGGGTCCAAGGTTGATACAG-3′. The PCR reaction was carried out in a 10-μl volume under the following conditions: 50 ng genomic DNA, 5 pmol of each primer, 1.5 mm $MgCl_2$, 0.2 mm of each dNTP, and 0.2 units Taq polymerase. A touchdown protocol was performed starting at 70° with 2° steps to the final annealing temperature of 60°. One microliter of the reaction volume was run on an automated fragment analysis system (A.L.F., Amersham) under denaturating conditions.

**Genotypes and haplotypes:** All genotypes were checked for Mendelian inheritance and double recombinants. Marker maps were calculated using the multipoint options of CRIMAP (version 2.4; Green *et al.* 1990). The most likely paternally and maternally inherited haplotypes for the four marker loci of the sons were estimated by a Markov chain Monte Carlo (MCMC) algorithm using the SIMWALK2 program haplotyping option (Sobel and Lange 1996). For these calculations, the genetic map as determined by CRIMAP was applied. Frequencies of alleles and haplotypes of the grandsires and frequencies of the maternally transmitted alleles and haplotypes of sons were obtained by allele/haplotype counting.

**Phenotypic data:** For our analyses daughter yield deviations (DYDs; Van Raden and Wiggans 1991) for milk yield, milk fat yield, milk protein yield, milk fat percentage, and milk protein percentage were used. DYDs were calculated by a test-day animal model from the first lactation data of daughters. DYDs for milk yield, milk fat yield, and milk protein yield were taken directly from the national breeding value evaluation for the Holstein breed in February 2003 (VIT, Verden, Germany). DYDs for milk fat percentage and milk protein percentage were calculated as described by Thaller *et al.* (2003).

**Association study at *DGAT1* promoter VTNR:** Standard QTL mapping (Knott *et al.* 1996) and testing (Churchill and Doerge 1994) procedures were applied to detect segregating sires that were homozygous for K232A at *DGAT1*. To further investigate the background of the genetic variance not explainable by the *DGAT1 K232A* mutation, we performed association

**TABLE 1**

**Results for the QTL analysis within families applied to milk fat percentage**

| Family | $n^a$ | Daughters[b] | *DGAT1 K232A*[c] | Position[d] | QTL effect[e] | $p_{chr}$[f] | $p_{chr}$Bonferroni[g] |
|---|---|---|---|---|---|---|---|
| 1 | 62 | 1961.0 | A/K | 1 | 0.203 | 0.00001 | 0.00032 |
| 2 | 58 | 577.3 | A/A | 5 | 0.059 | 0.14238 | 0.99266 |
| 3 | 29 | 650.7 | A/K | 1 | 0.225 | 0.00040 | 0.01272 |
| 4 | 123 | 1618.0 | A/A | 21 | 0.031 | 0.20916 | 0.99945 |
| 5 | 59 | 483.2 | A/A | 1 | 0.017 | 0.64496 | 1 |
| 6 | 30 | 2090.5 | A/K | 4 | 0.129 | 0.00621 | 0.18073 |
| 7 | 22 | 1909.2 | A/A | 1 | 0.051 | 0.58833 | 1 |
| 8 | 52 | 1072.2 | A/A | 21 | 0.059 | 0.14679 | 0.99378 |
| 9 | 18 | 335.4 | A/A | 1 | 0.084 | 0.54497 | 1 |
| 10 | 127 | 2152.1 | A/K | 1 | 0.185 | 0.00001 | 0.00032 |
| 11 | 124 | 409.1 | A/K | 1 | 0.205 | 0.00001 | 0.00032 |
| 12 | 28 | 621.9 | A/A | 16 | 0.086 | 0.20360 | 0.99931 |
| 13 | 32 | 176.6 | A/K | 1 | 0.126 | 0.01560 | 0.39537 |
| 14 | 57 | 1142.9 | A/A | 1 | 0.125 | 0.00645 | 0.18704 |
| 15 | 26 | 223.2 | A/A | 21 | 0.005 | 0.99458 | 1 |
| 16 | 41 | 311.0 | A/K | 1 | 0.098 | 0.04325 | 0.75703 |
| 17 | 60 | 1280.1 | A/K | 1 | 0.211 | 0.00001 | 0.00032 |
| 18 | 27 | 180.2 | A/A | 3 | 0.003 | 0.97721 | 1 |
| 19 | 48 | 81.8 | A/K | 1 | 0.210 | 0.00001 | 0.00032 |
| 20 | 75 | 222.0 | A/A | 1 | 0.196 | 0.00001 | 0.00032 |
| 21 | 42 | 96.3 | A/K | 1 | 0.163 | 0.00089 | 0.02809 |
| 22 | 20 | 53.2 | A/K | 1 | 0.062 | 0.48618 | 1 |
| 23 | 83 | 102.3 | A/A | 1 | 0.132 | 0.00010 | 0.00320 |
| 24 | 114 | 90.2 | A/A | 1 | 0.114 | 0.00002 | 0.00064 |
| 25 | 39 | 70.2 | A/K | 21 | 0.095 | 0.13566 | 0.99058 |
| 26 | 34 | 102.9 | A/K | 1 | 0.139 | 0.00445 | 0.13300 |
| 27 | 70 | 108.2 | A/A | 1 | 0.055 | 0.24787 | 0.99989 |
| 28 | 43 | 266.3 | A/A | 1 | 0.077 | 0.06691 | 0.89097 |
| 29 | 30 | 98.4 | A/A | 1 | 0.053 | 0.38402 | 1 |
| 30 | 55 | 99.2 | A/K | 9 | 0.147 | 0.00050 | 0.01588 |
| 31 | 56 | 91.7 | A/A | 1 | 0.219 | 0.08782 | 0.94721 |
| 32 | 11 | 62.7 | A/A | 4 | 0.072 | 0.46174 | 1 |
| 33 | 62 | 72.4 | A/A | | | | |
| 34 | 7 | 8119.3 | | | | | |

[a] Number of sons within families.
[b] Average number of daughters with milk records per son within family.
[c] *DGAT1 K232A* genotype of the grandsire of the respective family.
[d] Chromosomal position at the maximum of the test statistic relative to *DGAT1*.
[e] Estimates of the regression coefficients from the QTL analysis within family; QTL effects represent half of the chromosome substitution effect.
[f] Chromosome-wise type I error as determined by the permutation test.
[g] Chromosome-wise type I error as determined by the permutation test and after Bonferroni correction for testing multiple families.

studies including all *232A/232A* sons in the design. Initially, we tested for effects of *DGAT1* promoter VNTR alleles on milk fat percentage by multiple regression on the number of the respective VNTR alleles based on a substitution model (Mallard *et al.* 1995) using general linear model procedures (SAS procedure GLM; SAS Institute, Cary, NC),

$$y_{ij} = \mu + gs_i + b_1 z_{1ij} + b_2 z_{2ij} + b_3 z_{3ij} + b_4 z_{4ij} + b_5 z_{5ij} + e_{ij},$$

where $y_{ij}$ is the DYD of son $j$ within grandsire $i$, $\mu$ is the overall mean, $gs_i$ is the fixed effect of grandsire $i$, $z_{vij}$ is the number of alleles $v$ (1–5) at *DGAT1* promoter VNTR (0, 1, or 2) of son $j$ within grandsire $i$, $b_v$ is the regression coefficient of allele $v$, and $e_{ij}$ is the random residual effect. To avoid dependencies among equations, the effect of VNTR allele *5* was set to zero. Thus, the regression coefficients represent half of the allele substitution effect of VNTR allele $v$ when substituting VNTR allele *5*. The weight of each observation was proportional to one over the $\mathrm{Var}(e_{ij})$, $\mathrm{Var}(e_{ij})$ being

$$\mathrm{Var}(e_{ij}) = \left[\frac{3}{16}h^2 + \frac{(1 - (1/4)h^2)}{n_{ij}}\right]\sigma_P^2,$$

where $h^2$ is the heritability and $\sigma_P^2$ is the phenotypic variance of the trait investigated, respectively, and $n_{ij}$ is the number of daughters of son $j$ within sire $i$ included for the calculation of the DYD of son $j$.

The results from this analysis indicated allele *5* to have a positive effect on milk fat percentage compared to all other alleles. In addition, we observed that allele *5* was shared by all *232A/232A* grandsires segregating for milk fat percentage at the QTL.

Thus we conducted a subsequent association study by analyzing the effect of *DGAT1* promoter VNTR allele *5* compared to all other alleles. To estimate the size of an additive genetic effect of the *DGAT1* promoter VNTR allele *5*, a weighted regression analysis was performed applying the model

$$y_{ij} = \mu + gs_i + bz_{ij} + e_{ij},$$

where $y_{ij}$ is the DYD of son $j$ within grandsire $i$, $\mu$ is the overall mean, $gs_i$ is the fixed effect of grandsire $i$, $z_{ij}$ is the number of alleles *5* at *DGAT1* promoter VNTR (0, 1, or 2) of son $j$ within grandsire $i$, $b$ is the regression coefficient representing half of the allele substitution effect of VNTR allele *5* compared to all other alleles, and $e_{ij}$ is the random residual effect.

As the association analysis of the son's genotypes might be influenced strongly by the paternal alleles of the sons from their segregating *232A/232A* grandsires carrying *DGAT1* VNTR allele *5*, we performed an additional association test considering only the effect of the maternally inherited *DGAT1* promoter VNTR alleles. Thus we tested whether or not the assumed association could be confirmed at the level of bull dams. From the estimated most likely maternally inherited haplotypes, the maternally inherited *DGAT1* promoter VNTR allele was derived for the sons. These *DGAT1* promoter VNTR alleles were tested for association with milk production traits in the homozygous *232A/232A* sons of our data set by applying the model

$$y_{ijk} = \mu + gs_i + DGAT1\text{pro}_j + e_{ijk},$$

where $y_{ijk}$ is the trait value of son $k$ with maternally inherited VNTR allele $j$ within grandsire $i$, $\mu$ is the overall mean, $gs_i$ is the fixed effect of grandsire $i$, $DGAT1\text{pro}_j$ is the fixed effect of the maternally inherited *DGAT1* promoter VNTR allele $j$ (two classes were compared: allele *5 vs.* all other alleles), and $e_{ijk}$ is the random residual effect.

## RESULTS

**Distribution of alleles and haplotypes:** The frequencies of the marker alleles and haplotypes of the grandsires, as well as the frequencies of the maternally inherited marker alleles or haplotypes of sons, respectively, are listed in Tables 2 and 3. The most frequent alleles in both groups are the *DGAT1* promoter VNTR allele *3*, the *DGAT1* allele *232A*, the *ILSTS039* allele *1*, and the *CSSM066* allele *7*. However, for both *DGAT1* polymorphisms, the frequencies between the grandsires and the maternally inherited alleles of sons differed strongly.

The haplotype frequencies shown in Table 3 indicate linkage disequilibrium among the loci considered, especially between the *DGAT1* polymorphisms, with the *DGAT1* allele *232K* predominantly showing up with *DGAT1* promoter VNTR allele *3*. In contrast, the *DGAT1 232A* allele occurred on haplotypes together with all detected VNTR alleles. The almost fixed linkage phase between *DGAT1* promoter VNTR allele *3* and *DGAT1* allele *232K* prevented an association study of the *DGAT1* promoter VNTR variants in the sons with genotype *232K/232K* and also an investigation of possible interactions of the alleles at both *DGAT1* loci.

**QTL linkage analysis:** The regression analysis across families confirmed the segregation of a QTL for milk fat percentage at position 1 cM at the centromere of BTA14 ($F = 15.31$, $P < 0.00001$). This was expected, considering several previous mapping studies and because some of the sires were heterozygous at *K232A* in *DGAT1*. An association analysis across all sons in the data set (results not shown) confirmed the direction and size of highly significant effects of the *DGAT1 K232A* mutation on milk production traits found previously using a subset of our data set (Thaller *et al.* 2003). Genotyping of the *DGAT1 K232A* polymorphism identified 14 grandsires to be heterozygous *232A/232K* at K232A (Table 1), whereas 19 grandsires had genotype *232A/232A*. Linkage analysis within families revealed 5% chromosome-wise significant QTL effects in 12 families with grandsires heterozygous at *DGAT1 K232A* (Table 1). The 2 nonsegregating *232A/232K* grandsires (families 22 and 25) had only 20 and 39 sons, respectively, in the analysis and their DYDs were, on average, calculated from a relatively small number of daughters. More interesting, however, was the finding that 4 families with grandsires of genotype *232A/232A* showed a 5% chromosome-wise significant effect on milk fat percentage. The maximum of the test statistic in all 4 segregating families was achieved at position 1 cM (Table 1), which is consistent with mapping results in literature and corresponds to an assumed QTL position at the *DGAT1* locus or in its immediate vicinity. Segregating families with grandsires of *DGAT1* genotype *232A/232K* had a larger QTL effect, on average, compared to segregating families with grandsires of *DGAT1* genotype *232A/232A* (0.170% *vs.* 0.142% for milk fat percentage), although the difference was not significant statistically.

Applying a more stringent threshold by Bonferroni correction for testing multiple families still identified eight families with a *232A/232K* grandsire and three families with a *232A/232A* grandsire that segregated at a 5% significance level.

Two of the segregating *232A/232A* grandsires (families 23 and 24) were paternal half-sibs and showed similar QTL effects for their identical-by-descent (IBD) paternal haplotypes, which provides further confirmation for the segregation of a QTL in these families. The grandsire of family 20 (*VNTR 4-232A/VNTR 5-232A* genotype) was a son of the grandsire from family 10 (*VNTR 4-232A/VNTR 3-232K*) and received *VNTR 4-232A* from his father. In both families, the paternal chromosomal fragment carrying the *VNTR 4-232A* haplotype, which is identical by descent, was associated with a decreased milk fat percentage compared to the alternative paternal chromosomal fragment. Additionally, there was only a very small difference in the size of the QTL effect in both families despite the grandsires in the two families having different *DGAT1 K232A* genotypes.

**TABLE 2**

**Allele frequencies for *DGAT1* VNTR and *K232A* polymorphisms and for microsatellites *ILSTS039* and *CSSM066***

| | *DGAT1* promoter VNTR | | *DGAT1 K232A* | | *ILSTS039* | | *CSSM066* | |
|---|---|---|---|---|---|---|---|---|
| Allele | Grandsires | Maternal | Grandsires | Maternal | Grandsires | Maternal | Grandsires | Maternal |
| 1 | 0.014 | 0.017 | 0.279[a] | 0.491[a] | 0.500 | 0.520 | 0 | 0.002 |
| 2 | 0.222 | 0.169 | 0.721[b] | 0.509[b] | 0.152 | 0.082 | 0.012 | 0.014 |
| 3 | 0.292 | 0.477 | | | 0 | 0.002 | 0.110 | 0.035 |
| 4 | 0.250 | 0.175 | | | 0.273 | 0.243 | 0.098 | 0.133 |
| 5 | 0.222 | 0.163 | | | 0.015 | 0.044 | 0.231 | 0.136 |
| 6 | | | | | 0.030 | 0.092 | 0.061 | 0.086 |
| 7 | | | | | 0.030 | 0.017 | 0.317 | 0.430 |
| 8 | | | | | | | 0 | 0.004 |
| 9 | | | | | | | 0.159 | 0.147 |
| 10 | | | | | | | 0 | 0.003 |
| 11 | | | | | | | 0.012 | 0.008 |
| 12 | | | | | | | | 0.002 |

Allele frequencies were estimated on the basis of grandsires and on the basis of maternally inherited alleles of informative sons.

[a] Allele 1 represents *232K* at *DGAT1*.

[b] Allele 2 represents *232A* at *DGAT1*.

Fifteen out of the 16 grandsires with 5% chromosome-wise significant QTL effects were heterozygous at the *DGAT1* promoter VNTR. All segregating *DGAT1 232A/232A* grandsires were heterozygous at the *DGAT1* promoter VNTR and shared an identical genotype *4/5* at this locus. From the 14 *DGAT1 232A/232A* grandsires not segregating significantly at the 5% chromosome-wise level, 8 grandsires showed one allele *5* in their *DGAT1* promoter VNTR genotype. In seven of these families, the *DGAT1* promoter VNTR allele *5* was linked to the milk fat percentage-increasing QTL-allele, but contrasts between the *DGAT1* promoter VNTR allele *5* carrying paternal chromosomal segments and the alternative paternal segments were not significant. When comparing the QTL effect in families with grandsires having genotype *232A/232K*, the contrast of paternal chromosomes was lowest for families with grandsire VNTR genotype *3/5* and highest for families with grandsire genotype *3/4*. This indicates that the VNTR *5-232A*-carrying *DGAT1* haplotype is superior to the VNTR *4-232A*-carrying haplotype with respect to milk fat percentage, taking into account that the milk fat percentage-increasing *DGAT1 232K* allele was always associated with the VNTR allele *3*.

These results from QTL mapping indicated that the *DGAT1* VNTR allele *5* might be associated with an effect on milk fat percentage in addition to that of the *DGAT1 K232A* mutation. This hypothesis was tested in further analyses that considered the full genotypes of sons as well as their maternally inherited alleles.

**Association analyses of *DGAT1* promoter VNTR alleles:** Multiple regression analysis of *DGAT1* promoter VNTR alleles in sons of genotype *DGAT1 232A/232A* ($n = 550$) revealed that the allele substitution effect on milk fat content of allele *5* was higher than all other alleles (Table 4). The regression analysis of the *DGAT1* promoter VNTR alleles revealed significant effects for the *DGAT1* promoter VNTR allele *5* enhancing milk fat

**TABLE 3**

**Haplotype frequencies of four centromeric BTA14 loci (loci order: *DGAT1* promoter VNTR-*DGAT1 K232A-ILSTS039-CSSM066*)**

| Haplotype group[a] | Grandsire haplotypes | | Maternally inherited haplotypes | |
|---|---|---|---|---|
| | $n$[b] | Frequency[c] | $n$[b] | Frequency[c] |
| 1-A-*-* | 1 | 0.022 | 3 | 0.021 |
| 2-K-*-* | — | 0 | 2 | 0.006 |
| 2-A-1-3 | 1 | 0.109 | 1 | 0.051 |
| 2-A-2-9 | 1 | 0.109 | 1 | 0.065 |
| 2-A-*-* | 1 | 0.022 | 12 | 0.06 |
| 3-K-1-7 | 1 | 0.043 | 1 | 0.102 |
| 3-K-1-6 | 1 | 0.087 | 1 | 0.027 |
| 3-K-*-* | 3 | 0.065 | 9 | 0.078 |
| 3-A-*-* | 3 | 0.065 | 25 | 0.167 |
| 4-A-4-5 | 1 | 0.217 | 1 | 0.151 |
| 4-A-*-* | 2 | 0.043 | 7 | 0.067 |
| 5-A-1-7 | 1 | 0.152 | 1 | 0.129 |
| 5-A-4-* | 2 | 0.043 | 5 | 0.059 |
| 5-A-*-* | 1 | 0.022 | 5 | 0.019 |

Only haplotypes with frequencies >0.05 in at least one group were listed separately; all the other haplotypes were summarized according to the *DGAT1* promoter VNTR-*DGAT1 K232A* microhaplotypes.

[a] Variable alleles at the corresponding haplotype position are indicated by *.

[b] Number of haplotypes in the respective haplotype group.

[c] Haplotype frequencies of the respective haplotype or haplotype group.

**TABLE 4**

**Effects of *DGAT1* promoter VNTR alleles on milk fat percentage in *DGAT1 232A/232A* sons**

| *DGAT1* promoter VNTR allele | Allele frequency | α/2 (SE)[a] |
|---|---|---|
| *1* | 0.017 | −0.02584 (0.02571) |
| *2* | 0.217 | −0.02336 (0.0103)* |
| *3* | 0.161 | −0.01746 (0.01153) |
| *4* | 0.381 | −0.04259 (0.00892)** |
| *5* | 0.224 | 0 |

*$P < 0.05$; **$P < 0.001$.

[a] Half of the average allele substitution effect of the respective VNTR allele compared to allele *5* where α is defined according to Falconer and Mackay (1996).

percentage as well as milk protein percentage but decreasing milk yield and milk protein yield ($P = 0.063$; Table 5).

The association analysis of the maternally inherited *DGAT1* promoter VNTR alleles in sons ($n = 547$) also yielded significant effects of the *DGAT1* promoter VNTR allele *5* compared to all other VNTR alleles on milk fat percentage, milk yield, and milk protein yield (Table 6) similar in direction and size to the effects calculated in the analysis including both the maternal and paternal *DGAT1* promoter VNTR alleles (Table 5).

## DISCUSSION

**Detection of a third QTL allele at the *DGAT1* locus:** Previous publications report only two alleles at the QTL affecting milk fat percentage in the centromeric region of BTA14 (Spelman *et al.* 2002; Boichard *et al.* 2003). Spelman *et al.* (2002) replaced the *DGAT1* alleles *232K* and *232A* with the $QTL_{\text{milk fat percentage}}$ alleles *Q* and *q*, respectively, implying that the *DGAT1 K232A* mutation explains the QTL variation completely. In contrast, our results provide evidence for the segregation of at least three alleles affecting milk fat percentage at the *DGAT1* locus. This is based on three lines of evidence: (1) there is a significant QTL effect of the two alleles of the *DGAT1 K232A* mutation, (2) families with grandsires homozygous for the *DGAT1 K232A* mutation still show highly significant QTL effects, and (3) there is a significant association of *DGAT1* promoter VNTR alleles with milk fat percentage in sons with *DGAT1* genotype *232A/232A*. In particular, the significant segregation in families with grandsires of genotype *232A/232A* shows that genetic variation in the centromeric region of BTA14 exists beyond the *DGAT1 K232A* mutation. This indicates that there are $QTL_{\text{milk fat percentage}}$-increasing alleles *Q* within families associated with the *232A* allele at the *DGAT1 K232A* mutation. The analysis of QTL effects did not allow the unequivocal discrimination between QTL effects attributable to the *DGAT1 K232A* polymorphism and QTL effects due to other causative mutations. This was because the difference between the higher QTL effects in *232A/232K* grandsire families was not significantly different from the somewhat lower QTL effect in *232A/232A* grandsires. The genetic heterogeneity of the QTL for milk fat percentage corresponds to the estimation of Farnir *et al.* (2002), who used linkage disequilibrium mapping to calculate a heterogeneity parameter (proportion of *Q*-bearing alleles that are IBD with the founder chromosome-bearing marker allele contrasted to all other marker alleles) for the QTL of 0.81 for the Dutch Holstein population and 0.36 for the New Zealand Holstein population.

Our results may also explain why a combined linkage disequilibrium and linkage mapping approach to fine map the QTL in the centromeric region of BTA14 (Farnir *et al.* 2002) revealed several unexplainable results. For example, the analysis failed to obtain supplementary signals when accounting for the linkage disequilibrium component in a New Zealand granddaughter design after including maternal genetic information. Whether the segregation of more than two alleles at this QTL accounts for these problems remains to be investigated.

**TABLE 5**

**Effects of *DGAT1* promoter VNTR allele *5* on milk production traits in *DGAT1 232A/232A* sons**

| Trait | α/2[a] | SE | *P*-value[b] |
|---|---|---|---|
| Milk yield (kg) | −47.87 | ±19.26 | 0.013 |
| Milk fat percentage (%) | 0.0324 | ±0.0079 | <0.0001 |
| Milk fat yield (kg) | 0.414 | ±0.667 | 0.535 |
| Milk protein percentage (%) | 0.0080 | ±0.0040 | 0.046 |
| Milk protein yield (kg) | −0.980 | ±0.527 | 0.063 |

Average allele substitution effects of VNTR allele *5* compared to all other VNTR alleles.

[a] Half of the average allele substitution effect where α is defined according to Falconer and Mackay (1996).

[b] Statistical significance of the *DGAT1* VNTR effects.

**TABLE 6**

**Effects of the maternally inherited *DGAT1* promoter VNTR allele *5* on milk production traits in *DGAT1 232A/232A* sons**

| Trait | α/2[a] | SE | *P*-value[b] |
|---|---|---|---|
| Milk yield (kg) | −55.07 | ±26.06 | 0.035 |
| Milk fat percentage (%) | 0.0285 | ±0.0108 | 0.0083 |
| Milk fat yield (kg) | −0.131 | ±0.897 | 0.8838 |
| Milk protein percentage (%) | 0.0039 | ±0.0054 | 0.4756 |
| Milk protein yield (kg) | −1.502 | ±0.709 | 0.034 |

Average effects of VNTR allele *5* compared to all other VNTR alleles.

[a] Half of the average allele substitution effect where α is defined according to Falconer and Mackay (1996).

[b] Statistical significance of the *DGAT1* VNTR effects.

More than two QTL alleles at one locus may pose problems for linkage disequilibrium fine-mapping approaches that rely on a biallelic nature of the QTL.

The fact that some families are not segregating significantly despite being heterozygous at the commonly accepted causal *DGAT1 K232A* polymorphism or at the suggested VNTR polymorphism may be due to the conservative Bonferroni correction applied. Families included in the analysis are not independent and, probably more crucially, some of the youngest families comprise only a limited number of sons, thus restricting the power in detecting QTL.

In a previous study, polymorphisms of a bovine expressed sequence tag, KIEL_E8, showed an association with milk yield, milk fat yield, and milk protein yield in German Holsteins (Looft *et al.* 2001). Grisart *et al.* (2002) mapped the cysteine/histidine-rich protein (CHRP) gene that is homologous to KIEL_E8 to the immediate vicinity of the *DGAT1* gene in a BAC contig. Consequently, KIEL_E8 was a strong candidate for causing the genetic variation in addition to the *DGAT1 K232A* mutation. However, our analysis showed that the alleles of KIEL_E8 were in complete linkage disequilibrium with the *DGAT1 K232A* mutation in the grandsires of our data set, thus excluding KIEL_E8 variants as being causal for the genetic variance additional to the *DGAT1 K232A* mutation (data not shown).

**Association of genetic variance additional to *DGAT1 K232A* with *DGAT1* VNTR alleles:** For sons with homozygous *DGAT1* genotype *232A/232A*, allele *5* at the *DGAT1* promoter VNTR showed a significant effect on milk fat percentage in all analyses. The direction of the effect of the *DGAT1* promoter VNTR allele *5* on different milk production traits is identical to that of the *DGAT1 232K* allele (Spelman *et al.* 2002; Thaller *et al.* 2003), although the magnitude of the effect is much smaller. The estimates for the effects of the VNTR alleles are not in total agreement with the QTL effects found for segregating *232A/232A* sires. This might be due to either the well-known problem of overestimation of QTL effects in QTL mapping or to the presence of further variants beyond *K232A* and the VNTR polymorphisms. Nevertheless, the confirmation of the *DGAT1* promoter VNTR effect in the maternally inherited alleles shows that there should be an association between allele *5* at the *DGAT1* promoter VNTR and milk fat percentage at the population level. Whether allele *5* is a causal mutation or only in strong linkage disequilibrium with a new, currently unknown mutation in the vicinity of the *DGAT1* locus has to be investigated further. However, several lines of evidence indicate that the *DGAT1* promoter VNTR allele itself might influence milk fat synthesis, probably via the regulation of *DGAT1* expression.

**Indication of a causal effect of the *DGAT1* promoter VNTR alleles:** The variable number of repetitions of the 18mer element AGGCCCCGCCCTCCCCGG underlies the polymorphism in the *DGAT1* promoter VNTR. This 18mer element has not been detected in noncattle species (including human). However, it contains the CCCGCC motif, which is a potential binding site of the transcription factor SP1 (Alberts *et al.* 1989). In the eukaryotic genome, SP1 binding sites are well-known enhancer elements in genes with ubiquitous, yet inducible gene expression. In mice, *DGAT1* expression was detected in all samples of a large, comprehensive tissue collection (Cases *et al.* 1998). Therefore, a ubiquitous expression of the *DGAT1* gene may also be assumed in cattle, although bovine *DGAT1* expression has been documented only in the mammary gland (Grisart *et al.* 2002). In mammary gland epithelial cells, *DGAT1* has to be regulated according to the respective stage of lactation and therefore requires substantial gene induction. Members of the SP1 transcription factor family have been proposed to be required for the glucose-dependent induction of several glucose-responsive genes (Vaulon *et al.* 2000). Meegalla *et al.* (2002) showed that glucose enhances *DGAT1* mRNA expression, which would correspond to the presence of active SP1-binding sites in the *DGAT1* gene. The described characteristics of SP1-binding sites and *DGAT1* gene expression would suggest that the potential SP1-binding site cluster generated by the *DGAT1* promoter VNTR might well exert a regulatory function in the expression of the *DGAT1* gene. SP1-binding sites occur frequently in clusters. Mutation analysis of SP1-binding sites showed that the number of SP1-binding sites within a cluster could determine the transcription rate of the respective gene (*e.g.*, Yang *et al.* 1995). Therefore, the variability in the bovine *DGAT1* SP1 cluster, which is generated by the *DGAT1* promoter VNTR, could be responsible for variation in *DGAT1* expression and consequently of *DGAT1* activity in the mammary gland. Because *DGAT1* is known to be the key enzyme in controlling the synthesis rate of triglycerides in adipocytes, the level of *DGAT1* activity can be assumed to have a direct effect on milk fat content.

Further indicators of a causal effect of the *DGAT1* promoter VNTR alleles are given by a comprehensive mutation analysis of the *DGAT1* gene and its surrounding 5′ and 3′ genomic region. Winter *et al.* (2002) investigated several DNA pools of animals with extremely different performance regarding milk fat percentage and also DNA from several breeds and species. It is unlikely that a further polymorphism at the *DGAT1* locus with substantial variation in the Holstein breed would have been unnoticed in this study. We sequenced the grandsire of family 14, which showed a 5% chromosome-wise significant QTL for milk fat percentage, at all 19 polymorphic sites detected by Winter *et al.* (2002). With the exception of the *DGAT1* promoter VNTR, all other loci including the *DGAT1 K232A* mutation were homozygous in this grandsire.

The association of the *DGAT1* promoter VNTR alleles with milk production traits conforms to the hypothesis

that complex traits may result more frequently from noncoding regulatory variants than from coding sequence variants (Glazier *et al.* 2002). This indicates that polymorphisms in nontranslated regions of a candidate gene should not be rashly excluded as causative mutations underlying variability of complex traits in future investigations.

Any study to identify causal mutations at the *DGAT1* locus is hampered by the strong linkage disequilibria in the centromeric region of BTA14. These linkage disequilibria make the discrimination of trait-associated alleles from causal mutations very difficult using population level studies. For the *DGAT1* promoter VNTR variants, however, it will be possible to assess their direct effect on transcriptional regulation of the *DGAT1* gene by dissected promoter studies including only the targeted chromosomal region of the *DGAT1* gene. These experiments are under way.

**Distribution of *DGAT1* VNTR alleles:** The VNTR polymorphism at the *DGAT1* promoter shows a similar association pattern with the *DGAT1 K232A* mutation as do the microsatellite loci surrounding the *DGAT1* gene (Grisart *et al.* 2002). This distribution of extra- and intragenic repeat alleles is in contrast to the situation for the intragenic SNP alleles, which show only few haplotypes carrying the *232A* allele, but a variety of haplotypes carrying the *232K* allele (Grisart *et al.* 2002; Winter *et al.* 2002). The fixation of linkage phase for the haplotype carrying the ancient *DGAT1* allele *232K*, in contrast to a high variability of the *DGAT1* allele *232A*-carrying haplotypes, raises the question as to whether the fixation has functional relevance. If alleles at the *DGAT1* promoter VNTR have a phenotypic effect, then selection may have generated and maintained the fixation of the *DGAT1* haplotype VNTR allele *3-232K*. Another explanation for the almost-fixed linkage phase between allele *3* at the *DGAT1* promoter VNTR and the putative ancient *DGAT1 232K* allele, however, could be a strong founder effect in the Holstein breed. Tentative support for this hypothesis arises from haplotype analysis, which shows that the *DGAT1* allele *232K*-carrying haplotype found in the Fleckvieh breed (VNTR allele *4-DGAT1 232K*; Winter *et al.* 2002) has a very low frequency in the Holstein population. Spelman *et al.* (2002) described that the allele frequencies at *DGAT1 K232A* in the New Zealand Holstein subpopulations vary according to the proportion of sires imported from overseas, predominantly from the United States.

The population history of the German Holstein population suggests that any strong founder effect responsible for the fixed linkage phase between *DGAT1* allele *232K* and *DGAT1* promoter VNTR allele *3* in the German Holstein population might be due to a very strong North American introgression of the *232K* allele in the past 40 years. The difference in the allele frequency at the DGAT1 *K232A* mutation between the grandsires and the maternal alleles of sons in our data set as well as between cows and bulls in the Israeli Holstein population (Weller *et al.* 2003) is in agreement with the results of Thaller *et al.* (2003), who used a subset of our data set. Thaller *et al.* (2003) discussed that the difference in allele frequency might be due to the strong selection of the grandsires for high milk yield and milk protein yield favoring the *232A* allele on the one hand and different selection strategies with respect to milk fat percentage for bull dams on the other hand.

These results underscore that further investigations regarding evolution of allele frequencies in different cattle breeds and subpopulations and especially in the Holstein cattle population will be necessary to explain the present distribution and population dynamics of allele and haplotype frequencies at the *DGAT1* locus.

**Genetic heterogeneity at QTL loci:** The genetic heterogeneity at the *DGAT1* locus in cattle is analogous to the discovery of additional QTL alleles affecting meat quality in pigs at the *PRKAG3* locus (Ciobanu *et al.* 2001). Additionally, the analysis of the growth hormone receptor (GHR) gene, another putative causative gene for milk composition in cattle, indicated that there might be several alleles at this QTL on BTA20 as well. Blott *et al.* (2003) reported two sires that were homozygous for a mutation with strong association to milk composition, although they were heterozygous at the respective QTL. Our results are a further example of the hypothesis postulated by Jeon *et al.* (1999) and Nezer *et al.* (1999) that multiple alleles may be common at major gene loci in domestic animals under selection for many generations. Multiple alleles at the *DGAT1* locus may be one reason why genetic variation is maintained at this QTL with major effect on milk production traits, although phenotypic selection has been active for many generations.

From our results and the other examples of multiallelic QTL in the literature, we might hypothesize that QTL of complex traits might be determined frequently by the complex action of a variety of haplotypes comprising a number of mutations within or across genes. The detailed investigation of effects of single mutations as well as of the compound haplotypes will (1) provide basic knowledge about the impact of biochemical pathways and processes of gene regulation on the targeted complex biological traits within and across species and (2) also enable a more efficient implementation of the single mutations and/or haplotypes into marker-assisted selection in livestock production.

We thank the Vereinigte Informationssysteme für Tierhaltung w.V. (Verden) for providing pedigree information and DYDs. We also thank A. Garve, O. Haufft, and S. Wöhl for excellent technical assistance. Fruitful discussion with R. Weikard and R. Fürbass is thankfully acknowledged. I. Russ isolated the DNA for a subset of our pedigrees. This work was funded by the Federal Ministry of Education and Research (project 0311020A), the German Cattle Breeders Federation, and German artificial insemination stations.

## LITERATURE CITED


Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts *et al.*, 1989 *Molecular Biology of the Cell*, pp. 551–612. Garland Publishing, New York/London.

Blott, S., J. J. Kim, S. Moisio, A. Schmidt-Kuntzel, A. Cornet *et al.*, 2003 Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. Genetics **163:** 253–266.

Boichard, D., C. Grohs, F. Bourgeois, F. Cerqueira, R. Faugeras *et al.*, 2003 Detection of genes influencing economic traits in three French dairy cattle breeds. Genet. Sel. Evol. **35:** 77–101.

Cases, S., S. J. Smith, Y.-W. Zheng, H. M. Myers, S. R. Lear *et al.*, 1998 Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. Proc. Natl. Acad. Sci. USA **95:** 13018–13023.

Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

Ciobanu, D., J. Bastiaansen, M. Malek, J. Helm, J. Woollard *et al.*, 2001 Evidence for new alleles in the protein kinase adenosine monophosphate-activated $\gamma_3$-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics **159:** 1151–1162.

Coleman, R., and R. M. Bell, 1976 Triacylglycerol synthesis in isolated fat cells. Studies on the microsomal diacylglycerol acyltransferase. J. Biol. Chem. **251:** 4537–4543.

Dunner, S., M. E. Miranda, Y. Amigues, J. Canon, M. Georges *et al.*, 2003 Haplotype diversity of the myostatin gene among beef cattle breeds. Genet. Sel. Evol. **35:** 103–118.

Falconer, D. S., and T. F. C. Mackay, 1996 *Introduction to Quantitative Genetics*, Ed. 4. Longman Scientific & Technical, New York.

Farnir, F., B. Grisart, W. Coppieters, J. Riquet, P. Berzi *et al.*, 2002 Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. Genetics **161:** 275–287.

Glazier, A. M., J. H. Nadeau and T. J. Aitman, 2002 Finding genes that underlie complex traits. Science **298:** 2345–2349.

Green, P., K. Falls and S. Crooks, 1990 *Documentation of CRI-MAP*, Version 2.4. Washington University School of Medicine, St. Louis.

Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford *et al.*, 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.

Jeon, J. T., O. Carlborg, A. Tornsten, E. Giuffra, V. Amarger *et al.*, 1999 A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. Nat. Genet. **21:** 157–158.

Knott, S. A., J. M. Elsen and C. S. Haley, 1996 Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. **93:** 71–80.

Kühn, C., J. Bennewitz, N. Reinsch, N. Xu, H. Thomsen *et al.*, 2003 Quantitative trait loci mapping of functional traits in the German Holstein cattle population. J. Dairy Sci. **86:** 360–368.

Looft, C., N. Reinsch, C. Karall-Albrecht, S. Paul, M. Brink *et al.*, 2001 A mammary gland EST showing linkage disequilibrium to a milk production QTL on bovine chromosome 14. Mamm. Genome **12:** 646–650.

Ludwig, E. H., R. W. Mahley, E. Palaoglu, S. Ozbayrakci and M. E. Balestra, 2002 *DGAT1* promoter polymorphism associated with alterations in body mass index, high density lipoprotein levels and blood pressure in Turkish women. Clin. Genet. **62:** 68–73.

Mallard, B. A., K. E. Leslie, J. C. Dekkers, R. Hedge, M. Bauman *et al.*, 1995 Differences in bovine lymphocyte antigen associations between immune responsiveness and risk of disease following intramammary infection with Staphylococcus aureus. J. Dairy Sci. **78:** 1937–1944.

Meegalla, R. L., J. T. Billheimer and D. Cheng, 2002 Concerted elevation of acylcoenzyme A: diacylglycerol acyltransferase (DGAT) activity through independent stimulation of mRNA expression of DGAT1 and DGAT2 by carbohydrate and insulin. Biochem. Biophys. Res. Commun. **298:** 317–323.

Nezer, C., L. Moreau, B. Brouwers, W. Coppieters, J. Detilleux *et al.*, 1999 An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. Nat. Genet. **21:** 155–156.

Ogura, Y., D. K. Bonen, N. Inohara, D. L. Nicolae, F. F. Chen *et al.*, 2001 A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature **411:** 603–606.

Riquet, J., W. Coppieters, N. Cambisano, J. J. Arranz, P. Berzi *et al.*, 1999 Fine-mapping of quantitative trait loci by identity by descent in outbred populations: application to milk production in dairy cattle. Proc. Natl. Acad. Sci. USA **96:** 9252–9257.

Smith, S. J., S. Cases, D. R. Jensen, H. C. Chen, E. Sande *et al.*, 2000 Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. Nat. Genet. **25:** 87–90.

Sobel, E., and K. Lange, 1996 Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. Am. J. Hum. Genet. **58:** 1323–1337.

Spelman, R. J., C. A. Ford, P. McElhinney, G.C. Gregory and R.G. Snell, 2002 Characterization of the *DGAT1* gene in the New Zealand dairy population. J. Dairy Sci. **85:** 3514–3517.

Thaller, G., W. Krämer, A. Winter, B. Kaupe, G. Erhardt *et al.*, 2003 Effects of *DGAT1* variants on milk production traits in German cattle breeds. J. Anim. Sci. **81:** 1911–1918.

Thomsen, H., N. Reinsch, N. Xu, C. Looft, S. Grupe *et al.*, 2001 Comparison of estimated breeding values, daughter yield deviations and de-regressed proofs within a whole genome scan for QTL. J. Anim. Breed. Genet. **118:** 357–370.

Van Raden, P. M., and G. R. Wiggans, 1991 Derivation, calculation, and use of National Animal Model information. J. Dairy Sci. **74:** 2737–2746.

Vaulon, S., M. Vasseur-Cognet and A. Kahn, 2000 Glucose regulation of gene transcription. J. Biol. Chem. **275:** 31555–31558.

Weller, J. I., Y. Kashi and M. Soller, 1990 Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. J. Dairy Sci. **73:** 2525–2537.

Weller, J. I., M. Golik, E. Seroussi, E. Ezra and M. Ron, 2003 Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population J. Dairy Sci. **86:** 2219–2227.

Winter, A., W. Kramer, F. A. O. Werner, S. Kollers, S. Kata *et al.*, 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. Proc. Natl. Acad. Sci. USA **99:** 9300–9305.

Yang, X., D. Fyodorov and E. S. Deneris, 1995 Transcriptional analysis of acetylcholine receptor α3 gene promoter motifs that bind Sp1 and AP2. J. Biol. Chem. **270:** 8514–8520.

Yu, Y. H., Y. Y. Zhang, P. Oelkers, S. L. Sturley, D. J. Rader *et al.*, 2002 Posttranscriptional control of the expression and function of diacylglycerol acyltransferase-1 in mouse adipocytes. J. Biol. Chem. **277:** 50876–50884.


Communicating editor: C. Haley