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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[1]

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ABSTRACT: Recent publications indicate genetic variation in milk production traits on proximal BTA14, which cannot be explained solely with genetic variation in the DGAT1 gene. To elucidate these QTL effects, animals from a German Holstein granddaughter design (18 families, 1,291 sons) were genotyped for CYP11B1 (V30A) and DGAT1 (K232A) polymorphisms. Frequencies of alleles of maternal descent were estimated for $CYP11B1^V$ (0.776) and $DGAT1^K$ (0.549). Allele substitution effects (α/2) were first calculated for both alleles in separate models and then in a joint model. From the joint analysis, $CYP11B1^V$ effects on fat content (+0.04%) and protein content (+0.01%) were positive. Effects on milk yield (–82 kg), fat yield (–0.5 kg), and protein yield (–1.9 kg) were negative. Compared with the individual analysis, $DGAT1^K$ effects on fat content (+0.28%), protein content (+0.06%), and milk yield (–258 kg) were reduced; fat yield (+10.8 kg) was enhanced; and protein yield (–3.8 kg) was reduced. In the joint analysis, allele substitution effects of $CYP11B1^V$ and $DGAT1^K$ together explained more of the variation in milk production traits than $DGAT1^K$ alone. Further significant effects were found for $CYP11B1^V$ and $DGAT1^K$ among 6 reproduction traits and 14 conformational traits. These observations indicate a possible negative influence of $DGAT1^K$ on maternal nonreturn rate, and thus, on length of productive life.

Key words: BTA14, CYP11B1, DGAT1, milk production trait, quantitative trait locus

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INTRODUCTION

Bovine chromosome 14 (BTA14) has been the subject of research for QTL of dairy-related traits for a long time. In a meta-analysis Khatkar et al. (2004) summarized 11 reports indicating the presence of a QTL for fat content and milk yield on proximal BTA14. The DGAT1 gene was identified as a candidate gene for this QTL (Grisart et al., 2002; Winter et al., 2002). Bennewitz et al. (2004) and Fisher and Spelman (2004) conducted an investigation into the putative QTL. When taking DGAT1 data into account they still found evidence for further genetic variation on proximal BTA14 for milk production traits.

The 11β-hydroxylase (CYP11B1) is the enzyme (EC:1.14.15.4) that catalyzes both the 11β- and 18-hydroxylation of corticosteroids in cattle (Ogishima et al., 1989; Muller, 1998; Lisurek and Bernhardt, 2004). Steroid hormones are physiological regulators and cortisol is one of the principal hormones involved in lipogenesis and lipolysis, which control fatty acid concentration in plasma and tissues (Bhathena, 2000). The CYP11B1 hormones influence fluid volume and electrolyte homeostasis and glucose and lipid metabolism (Kirita et al., 1990; Bülow and Bernhardt, 2002).

In some species, the CYP11B1 gene has developed into distinct isoforms (Kawamoto et al., 1992; Mellon et al., 1995; Bülow et al., 1996; Muller, 1998), whereas in pig, sheep, and cattle functional unity is conserved (Bülow et al., 1996; Muller, 1998). In all mammals CYP11B1 pseudogenes exist (Kirita et al., 1990; Mellon et al., 1995). Because the CYP11B1 coding gene has been mapped to BTA14q12 (Kaupe et al., 2004a) and HSA8q21-23 (Wagner et al., 1991; Taymans et al., 1998), this gene can be considered as a positional candi-

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date gene. Because CYP11B1 is involved in energy metabolism, this gene can also be considered as a functional candidate gene for milk production.

The objective of this study was to investigate the effect of a CYP11B1 polymorphism on the QTL for milk production traits, which could not be completely resolved with DGAT1 variation.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not obtained for this study because the data were obtained from an existing database (United Data Systems for Animal Production, VIT, Verden, Germany).

Population Structure

The granddaughter design for Holstein cattle of the German Cattle Breeders Association (ADR, Bonn, Germany) has been described by Thomsen et al. (2000). Different authors have characterized this granddaughter design for milk production traits (Thomsen et al., 2000; Bennewitz et al., 2003, 2004; Thaller et al., 2003), functional traits (Kühn et al., 2003), and conformation and behavioral traits (Hiendleder et al., 2003). From this population, DNA of 1,291 animals from 18 paternal half-sib families was genotyped. The number of sons per Holstein sire ranged from 19 to 352, with 69.4 sons per family on average. Sons were born between 1986 and 1993. In addition, 202 German Simmental (Fleckvieh) animals were also genotyped.

Molecular Analysis of CYP11B1 Polymorphism

To develop primers, advantage was taken of a deletion of 4 nucleotides (GAGG) in the 5′-region of the CYP11B1 pseudogene sequence (Kirita et al., 1990; NCBI trace archive: 380062637, 656145983, 884919011), which correspond to positions –263, –264, –265, and –266 in the 5′-sequence of the coding CYP11B1 gene, GenBank mRNA sequence NM_174638 (Morohashi et al., 1987). Using the actual sequence adjacent to the deletion, 2 primers (245, 251) were designed that had 3 or 4 of the deleted nucleotides (underlined, bold letters), respectively, as their terminal 3′-nucleotides. As a control group, 2 more primers (255, 256) were designed that did not contain the deleted nucleotides and that were meant to anneal to the CYP11B1 pseudogene only. This procedure led to 4 forward primers:

245, 5′-ATACTGGAGGGGGAG**GAGG**-3′;
251, 5′-CATACTGGAGGGGGAG**GAG**-3′;
255, 5′-CATACTGGAGGGGGAGCCTC-3′; and
256, 5′-ACTGGAGGGGGAGCCTCTTG-3′.

These forward primers were employed in separate PCR reactions together with the same reverse primer (195):

195, 5′-GGACAGAACGTGAGGGTGTT-3′;

which is located at the beginning of the intron 1 sequence. The primer pairs were expected to yield 2 distinct fragments of differing size (245/195, 568 bp; and 251/195, 570 bp) from the coding gene and 2 fragments (255/195, 565 bp; and 256/195, 562 bp) from the pseudogene.

The PCR was performed in a GeneAmp PCR System 9600 (Applied Biosystems, Foster City, CA) using a 20 μL volume containing 50 ng of genomic DNA, 0.5 U of Hotstar Taq (Qiagen, Hilden, Germany), 1× Hotstar PCR Buffer, 1.5 m*M* $MgCl_2$, 150 μ*M* each of dNTP, 0.5 p*M* each of forward and reverse primer, and 5% DMSO because of a GC-rich (66%) PCR product. The PCR profile included 1 cycle of 15 min at 95°C, followed by 10 cycles of 30 s at 94°C, 30 s at 72°C to 62°C (1°C less every cycle) and 45 s at 72°C, followed by 30 cycles of 30 s at 94°C, 30 s at 62°C, and 45 s at 72°C, followed by 1 cycle of 10 min at 72°C.

The PCR products were run on a polyacrylamide gel to detect conformational differences in the amplified molecules, at least between those from the coding CYP11B1 gene and the CYP11B1 pseudogene. Therefore, PCR products were denatured for 1 min at 90°C in a 95% formamide dye and subsequently chilled in ice water. Fragments were then separated by electrophoresis on a nondenaturing 10% polyacrylamide gel with 1% glycerol, 0.5× TBE at 5°C, 650 V for 5 h in a P9DS Penguin system (OWL Separation Systems, Portsmouth, NH). Gels were then fixed in 10% acetic acid:15% ethanol (vol/vol), and silver-stained, essentially following the procedure of Bassam et al. (1991), but using 0.04 *M* EDTA as the stop solution.

As further evidence for selectivity of the primer systems, PCR products were sequenced on an ABI 377 PRISM sequencer (Applied Biosystems, Foster City, CA) after cleanup of the PCR products with a Montage PCR Centrifugal Filter device (Millipore, Eschborn, Germany) and purification of the sequencing reactions with a DyeEx 2.0 Spin Kit (Qiagen, Hilden, Germany).

For genotyping, primer pair 245/195 was selected to amplify the DNA from all 1,291 animals with PCR, as described above. The PCR products of 568 bp were digested with the restriction enzyme PstI (MBI Fermentas, St Leon-Rot, Germany) in 20-μL volumes overnight. Electrophoresis was carried out with 2% agarose gels and 4 V/cm in 0.5× TBE buffer. After differential migration of digests on the genotyping gel, the fragment set of 442/103/23 bp represented the valine variant ($CYP11B1^V$) and the fragment set of 371/103/71/23 bp represented the alanine variant ($CYP11B1^A$) of the CYP11B1 coding gene.

Molecular Analysis of DGAT1 Polymorphism

A 411-bp fragment of the bovine DGAT1 gene containing the K232A substitution was amplified by PCR and digested with the restriction enzyme CfrI (MBI

Fermentas) as reported recently (Kaupe et al., 2004b). Electrophoretic separation on agarose gels identified phenotypes by differential migration due to fragment size. The uncut 411-bp fragment represented the lysine variant ($DGAT1^K$), whereas digested fragments of 203 and 208 bp represented the alanine variant ($DGAT1^A$) of the DGAT1 gene.

Phenotypic Data

Phenotypic data used in this study included EBV (Liu et al., 2000a,b), reliabilities (Liu et al., 2001), and number of daughters for all German Holstein sires. These were obtained from the United Data Systems for Animal Production (VIT, Verden, Germany) and included EBV for milk yield, fat and protein yield, and fat and protein content. Also included were EBV of somatic cell score (**SCS**) and 14 conformational traits: dairy character, stature, body depth, strength, rump angle, rump width, rear leg set side view, foot angle, rear udder height, suspensory ligament, teat placement, fore udder attachment, teat length, and udder depth. Six reproduction traits: paternal calving ease, paternal stillbirth, maternal calving ease, maternal stillbirth, paternal nonreturn rate, and maternal nonreturn rate, were also considered, together with the EBV for functional herd life. Production and conformation data were taken from the national breeding value evaluation of May 2004; fertility data were based on the August 2003 evaluation. Evaluation methods and statistical models of breeding values for milk production traits, SCS, conformation, productive life, and reproduction traits in German Holstein cattle can be consulted online (http://www.vit.de/; last accessed 21 September 2006).

Statistical Analysis

Allele frequencies of $CYP11B1^{V+A}$ and $DGAT1^{K+A}$ were estimated from alleles of maternal descent. With progeny of homozygous sires, frequencies were directly deduced from the genotypes of the sons. With offspring from heterozygous sires, alleles of maternal descent could only be determined unequivocally if the sons were homozygous. Therefore, a maximum likelihood procedure was applied for estimating allele frequencies:

$$\hat{p} = \frac{n'_{11} + n_{12}}{n'_{11} + n_{12} + n'_{22} + n_{22}},$$

where $\hat{p}$ represents the allelic frequency of $CYP11B1^V$ (V = 1, A = 2) or $DGAT1^K$ (K = 1, A = 2); n'_{11} and n'_{22} are the number of homozygous (11/22) sons within heterozygous sires, respectively; and n_{12} and n_{22} are the numbers of heterozygous (12) and homozygous (22) sons within homozygous (22) sires, respectively. The total number of sons with alternative genotypes across all homozygous and all heterozygous sires, respectively, was used for estimating allele frequencies.

The de-regressions of estimated breeding values (**DRBV**) for all traits were calculated by applying an iterative procedure, as described by Jairath et al. (1998), which is currently used by Interbull (Uppsala, Sweden). The differing number of daughters per sire contributing to the calculation of DRBV was accounted for by a weighting factor, W:

$$W = \frac{n}{1 + (n - 1)\frac{1}{4}h^2},$$

in which the number of effective daughters of each sire contributing to the calculation of the DRBV equals n, and h^2 corresponds to the heritability of the respective trait. For reproduction traits, the number of daughters was not available. De-regressed proofs for these traits were calculated applying the formula described by Kühn et al. (2003), together with an unweighted analysis.

Data were analyzed using the GLM procedure (SAS Inst., Inc., Cary, NC). The gene substitution effects (α/2), as defined in Falconer and MacKay (1996), for $CYP11B1^V$ and $DGAT1^K$ were estimated with the following fixed models:

Fixed model for an individual analysis of CYP11B1 effects, $y_{ij} = \mu + sire_i + b_1 \cdot x_{ij} + e_{ij}$;
Fixed model for an individual analysis of DGAT1 effects, $y_{ij} = \mu + sire_i + b_2 \cdot z_{ij} + e_{ij}$; and
Fixed model for a joint analysis of both CYP11B1 and DGAT1 effects, $y_{ij} = \mu + sire_i + b_1 \cdot x_{ij} + b_2 \cdot z_{ij} + e_{ij}$;

where y_{ij} is the DRBV of son j within sire i, μ is the overall mean, $sire_i$ is the fixed effect of sire i, x_{ij} is the number of *CYP11B1*^{V} alleles (0, 1, or 2 for *CYP11B1*^{V}), z_{ij} is the number of *DGAT1*^{K} alleles (0, 1, or 2 for *DGAT1*^{K}) of son j within sire i, b_1 and b_2 are the regression coefficients representing half of the gene substitution effect (α/2), and e_{ij} is the random residual effect including polygenic and environmental effects. Sires were included as fixed effects because they represent highly selected animals from the sire population and cannot be considered as a random sample.

RESULTS

Molecular Investigation

On the basis of a deletion of 4 nucleotides (GAGG) in the bovine CYP11B1 pseudogene sequence, 4 primer pairs (245 / 195, 251 / 195, 255 / 195, 256 / 195) amplified selectively the 5′- and exon 1 regions of the coding gene or pseudogene of bovine CYP11B1. Selectivity was verified by polyacrylamide gel electrophoresis. There, using DNA from 5 different animals, PCR products 245 / 195 produced 3 migration patterns (AA, AB, BB) and PCR-products 256 / 195 showed only 1 invariant pattern (data not shown). The presence of the 4 nucleotides

Table 1. Nucleotide substitutions in the 5′-region and exon 1 of bovine *CYP11B1* between the coding gene and the pseudogene sequence, which were derived from PCR products amplified with specific primers for both gene variants

Nucleotide position[1]	*CYP11B1* (5′-region and exon 1)	
	Coding	Pseudo
–266	G	—
–265	A	—
–264	G	—
–63	G	—
–212	C	T
–211	A	G
–157	G	C
–62	C	T
+59	A	G
+65	G	A
+68	T	C
+89	T	C
+114	C	T
+180	T	C

[1]— = 5′-end sequence position counted antisense. + = gene sequence position beginning with A = 1 (initiation codon).

(GAGG) in PCR fragment 245 / 195, and the lack of these nucleotides in PCR fragment 256 / 195 was confirmed by ABI377 sequencing.

Table 1 includes all nucleotide substitutions found in coding and pseudo sequences. The digestion of PCR products of the CYP11B1 gene with restriction enzyme PstI, and the DGAT1 gene with restriction enzyme CfrI showed a clear separation of the 3 genotypes (AA, AB, BB).

Descriptive Statistics of De-Regressed Breeding Values

De-regressed breeding values of production traits, fertility traits, and conformation are given in Table 2. Average DRBV for yield traits and SCS were positive, those for percentage traits were negative. Heritabilities were taken from the EBV-publication (VIT, Verden, Germany), which can be consulted online (http://www.vit.de/). Reliabilities were above 85% on average for most traits.

Allelic and Genotypic Frequencies

Differences in the occurrence and frequency of different alleles in CYP11B1 and DGAT1 genes could be observed, but to a larger extent for DGAT1 than for CYP11B1. Over all families in the German Holstein granddaughter design, allele frequencies were 0.776 for $CYP11B1^{V}$ and 0.549 for $DGAT1^{K}$. An additional typing of 202 German Simmental (Fleckvieh) cattle (data not shown) gave a frequency of 0.728 for $CYP11B1^{V}$ for this breed. As shown in Table 3, out of 18 Holstein sires, 9 were heterozygous for $CYP11B1^{V+A}$ and 9 were heterozygous for $DGAT1^{K+A}$ alleles. Only 5 sires were heterozygous for both polymorphisms. Nine sires were homozygous for $CYP11B1^{V}$; none were homozygous for $CYP11B1^{A}$; 8 were homozygous for $DGAT1^{A}$; and 1 was homozygous for $DGAT1^{K}$. Out of 1,291 German Holstein sons, 48% were homozygous for $CYP11B1^{V}$, 44% heterozygous for $CYP11B1^{A+V}$, and 8% homozygous for $CYP11B1^{A}$, whereas 16% were homozygous for $DGAT1^{K}$, 51% heterozygous for $DGAT1^{A+K}$, and 33% homozygous for $DGAT1^{A}$.

Effects of $CYP11B1^{V}$ and $DGAT1^{K}$

In all regression models applied to estimate effects of the $CYP11B1^{V}$ and $DGAT1^{K}$ alleles, the fixed effect of sire was significant. Table 4 shows estimated effects ($\alpha/2$) of $CYP11B1^{V}$ and $DGAT1^{K}$ with their respective standard errors for milk production and fertility traits. Effects of $CYP11B1^{V}$ and $DGAT1^{K}$ on milk production traits were all highly significant ($P < 0.01$). The $CYP11B1^{V}$ was significant ($P < 0.05$) for SCS when calculating regression coefficients for each gene separately. However, in a joint analysis, uniting both alleles in 1 model, results changed for $CYP11B1^{V}$, whereas $DGAT1^{K}$ remained highly significant ($P < 0.01$) for all 5 production traits. The $CYP11B1^{V}$ effects on milk yield ($P < 0.01$), fat content ($P < 0.01$), and protein yield ($P < 0.01$) again were highly significant, whereas effects on protein content ($P < 0.05$) were significant. The effect on fat yield lost significance.

Analysis of fertility traits in German Holsteins showed significant effects for $CYP11B1^{V}$ on paternal calving ease ($P < 0.05$) and for $DGAT1^{K}$ on maternal nonreturn rate ($P < 0.05$) when estimated separately. In the joint model, $DGAT1^{K}$ remained significant ($P < 0.05$) for maternal nonreturn rate, but $CYP11B1^{V}$ was highly significant ($P < 0.01$) for paternal calving ease.

Analysis of conformational traits (Table 5) using separate models for $CYP11B1^{V}$ and $DGAT1^{K}$ showed no significant values for $CYP11B1^{V}$. The $DGAT1^{K}$ allele had highly significant effects on strength ($P < 0.01$) and significant effects on rump width ($P < 0.05$). Repeating the estimation in the joint model yielded similar $\alpha/2$ values for $DGAT1^{K}$. The $CYP11B1^{V}$ effects remained without significance in this model.

Correlations Among De-Regressed Breeding Values

Correlations among DRBV were calculated for fertility and milk production traits on one hand, and length of productive life, SCS, and milk production traits on the other (Table 6). Paternal calving ease was negatively correlated with milk yield ($P < 0.05$), fat yield ($P < 0.01$), protein yield ($P < 0.05$), and SCS ($P < 0.01$). Paternal stillbirth was positively correlated with protein yield ($P < 0.01$) and negatively with SCS ($P < 0.01$). Maternal nonreturn rate 90 was negatively correlated with milk yield ($P < 0.05$), fat yield ($P < 0.001$), protein yield ($P < 0.001$), and fat percent ($P < 0.05$). Length of

Table 2. Number of sons, mean, SD, minimum, and maximum of de-regressed breeding values (DRBV), heritabilities (h^2), and average reliabilities (a.r.) for milk production traits, fertility traits, and conformational traits of the granddaughter-design of German Holstein cattle

Trait DRBV	No.	Mean	SD	Minimum	Maximum	h^2	a. r.
Milk yield, kg	1,290	+273	553	–3,237	+1,998	0.35	96.0
Fat yield, kg	1,290	+5.92	20.62	–79.23	+73.65	0.36	96.0
Protein yield, kg	1,290	+6.87	15.62	–85.46	+59.39	0.38	96.0
Fat content, %	1,291	–0.06	0.30	–0.98	+1.37	0.35	96.0
Protein content, %	1,291	–0.03	0.12	–0.59	+0.35	0.35	96.0
Somatic cell score	1,288	+0.04	0.51	–0.41	+2.60	0.16	92.5
Paternal calving ease	1,267	0.00	0.05	–0.40	+0.22	0.05	85.6
Maternal calving ease	1,287	–0.01	0.06	–0.30	+0.21	0.05	72.7
Paternal stillbirth	1,267	–1.00	2.40	–15.15	+8.00	0.05	85.6
Maternal stillbirth	1,267	+0.78	2.94	–11.50	+10.53	0.05	72.7
Paternal nonreturn rate 90	1,193	–1.18	6.01	–39.85	+22.54	0.02	66.1
Maternal nonreturn rate 90	1,283	–1.15	6.00	–20.59	+18.91	0.02	62.1
Length of productive life	1,286	+39.85	192.92	–162.20	+773.49	0.16	84.4
Dairy character	1,279	+95.12	16.42	–21.42	+198.36	0.24	83.9
Stature	1,283	+93.71	14.33	+41.55	+152.83	0.41	89.4
Body depth	1,283	+97.74	16.01	+38.00	+150.20	0.24	84.0
Strength	1,279	+97.42	18.74	–38.24	+238.79	0.18	81.1
Rump angle	1,283	+97.71	16.31	+34.65	+153.34	0.26	85.1
Rump width	1,283	+97.64	15.28	+30.68	+144.81	0.28	85.6
Rear leg set side view	1,283	+99.90	20.21	–86.64	+223.46	0.15	79.7
Foot angle	1,282	+96.16	21.90	–40.46	+192.13	0.12	77.1
Rear udder height	1,283	+95.56	17.07	+32.43	+170.28	0.22	83.2
Suspensory ligament	1,283	+100.21	22.73	–160.57	+176.09	0.13	78.2
Teat placement	1,285	+94.92	17.72	0.00	+150.31	0.22	83.4
Fore udder attachment	1,282	+96.71	17.10	+8.00	+150.76	0.21	82.8
Udder depth	1,285	+101.04	15.70	0.00	+147.18	0.26	85.1
Teat length	1,283	+100.46	16.17	+50.58	+166.37	0.25	84.7

Table 3. Number of sons per grandsire and distribution of valine to alanine substitution (V30A) genotypes at the gene encoding 11β-hydroxylase ([EC1.14.15.4]; *CYP11B1*) and lysine to alanine substitution (K232A) genotypes at the gene encoding diacylglycerol O-acyltransferase ([EC2.3.1.20]; *DGAT1*) within each grandsire family of the German Holstein granddaughter design

Grandsire family[3]	n	*CYP11B1* Genotype[1]			*DGAT1* Genotype[2]		
		VV	VA	AA	KK	KA	AA
01	32	23	9	0	**8**	**13**	**11**
02	42	**16**	**21**	**5**	0	19	23
03	22	**8**	**11**	**3**	0	10	12
04	51	39	12	0	0	36	15
05	18	13	5	0	0	10	8
06	128	**53**	**61**	**14**	**38**	**60**	**30**
07	275	**115**	**133**	**27**	**78**	**137**	**60**
08	28	17	11	0	0	17	11
09	23	17	6	0	**4**	**10**	**9**
10	31	22	9	0	**9**	**12**	**10**
11	56	**29**	**26**	**1**	0	27	29
12	29	**14**	**12**	**3**	**11**	**13**	**5**
13	19	15	4	0	11	8	0
14	25	19	6	0	0	14	11
15	42	31	11	0	**10**	**20**	**12**
16	352	**123**	**181**	**48**	0	188	164
17	60	**32**	**26**	**2**	**19**	**29**	**12**
18	58	**30**	**27**	**1**	**17**	**30**	**11**

[1]CYP11B1 variants are represented by letters: V = valine, and A = alanine.
[2]DGAT1 variants are represented by letters: K = lysine, and A = alanine.
[3]Details of sons of heterozygous (KA or VA) sires are in boldface.

Table 4. Regression coefficients for the number of copies of the valine allele (*CYP11B1*^{V}) and lysine allele (*DGAT1*^{K}) representing half of the allele substitution effects ($\alpha/2$)[1], SE, and R^2 for milk production traits and fertility traits in German Holstein cattle, as individually and jointly detected

	Individual analysis				Joint analysis		
Trait DRBV[2]	*CYP11B1*^{V}		*DGAT1*^{K}		*CYP11B1*^{V}	*DGAT1*^{K}	
Milk production trait	$\alpha/2$ ± SE	R^2	$\alpha/2$ ± SE	R^2	$\alpha/2$ ± SE	$\alpha/2$ ± SE	R^2
Milk yield, kg	–182 ± 23***	0.14	–288 ± 22***	0.20	–82 ± 24***	–258 ± 24***	0.21
Fat yield, kg	3.66 ± 0.88***	0.15	10.64 ± 0.81***	0.24	–0.51 ± 0.90	10.83 ± 0.87***	0.24
Protein yield, kg	–3.36 ± 0.66***	0.15	–4.48 ± 0.64***	0.16	–1.89 ± 0.71**	–3.79 ± 0.69***	0.17
Fat content, %	0.15 ± 0.01***	0.20	0.29 ± 0.01***	0.50	0.04 ± 0.01***	0.28 ± 0.01***	0.50
Protein content, %	0.04 ± 0.01***	0.15	0.07 ± 0.01***	0.24	0.01 ± 0.01*	0.06 ± 0.01***	0.24
Somatic cell score	0.05 ± 0.02*	0.19	0.03 ± 0.02	0.19	0.04 ± 0.02	0.01 ± 0.02	0.19
Fertility trait	$\alpha/2$ ± SE	R^2	$\alpha/2$ ± SE	R^2	$\alpha/2$ ± SE	$\alpha/2$ ± SE	R^2
Paternal calving ease	5.10E–3 ± 2.30E–3*	0.08	–1.23E–3 ± 2.20E–3	0.08	6.49E–3 ± 2.43E–3**	–3.60E–3 ± 2.36E–3	0.08
Maternal calving ease	3.89E–3 ± 2.69E–3	0.04	0.62E–3 ± 2.60E–3	0.04	–4.79E–3 ± 2.90E–3	2.35E–3 ± 2.81E–3	0.04
Paternal stillbirth	–0.09 ± 0.11	0.05	–0.11 ± 0.11	0.05	–0.06 ± 0.12	–0.09 ± 0.11	0.05
Maternal stillbirth	0.09 ± 0.13	0.10	0.08 ± 0.13	0.10	0.07 ± 0.14	0.06 ± 0.14	0.10
Paternal nonreturn 90	–0.23 ± 0.29	0.03	–0.30 ± 0.28	0.03	–0.13 ± 0.31	–0.25 ± 0.30	0.03
Maternal nonreturn 90	–0.05 ± 0.26	0.09	–0.53 ± 0.26*	0.09	0.18 ± 0.29	–0.59 ± 0.28*	0.09
Functional trait	$\alpha/2$ ± SE	R^2	$\alpha/2$ ± SE	R^2	$\alpha/2$ ± SE	$\alpha/2$ ± SE	R^2
Length of productive life	–3.85 ± 8.55	0.10	–2.63 ± 8.30	0.10	–3.30 ± 9.22	–1.43 ± 8.95	0.10

[1]α as defined in Falconer and MacKay (1996).
[2]DRBV = De-regressed breeding values.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 5. Regression coefficients for the number of copies of the valine allele (*CYP11B1*^{V}) and lysine allele (*DGAT1*^{K}) representing half of the allele substitution effects ($\alpha/2$)[1], SE, and R^2 for conformational traits in German Holstein cattle, as individually and jointly detected

	Individual analysis				Joint analysis		
Trait DRBV[2]	*CYP11B1*^{V}		*DGAT1*^{K}		*CYP11B1*^{V}	*DGAT1*^{K}	
Conformational trait	$\alpha/2$ ± SE	R^2	$\alpha/2$ ± SE	R^2	$\alpha/2$ ± SE	$\alpha/2$ ± SE	R^2
Dairy character	–0.04 ± 0.68	0.10	0.86 ± 0.66	0.10	–0.43 ± 0.74	1.02 ± 0.72	0.10
Stature	–0.23 ± 0.64	0.07	–0.54 ± 0.62	0.08	–0.02 ± 0.69	–0.53 ± 0.67	0.08
Body depth	–0.34 ± 0.66	0.18	–0.75 ± 0.64	0.18	–0.06 ± 0.71	–0.73 ± 0.69	0.18
Strength	–0.41 ± 0.76	0.08	–2.07 ± 0.74**	0.08	0.45 ± 0.81	–2.23 ± 0.79**	0.08
Rump angle	–0.95 ± 0.68	0.16	–0.98 ± 0.66	0.16	–0.67 ± 0.74	–0.74 ± 0.72	0.16
Rump width	0.27 ± 0.65	0.12	–1.34 ± 0.63*	0.13	0.91 ± 0.70	–1.67 ± 0.68*	0.13
Rear leg set side view	0.86 ± 0.84	0.05	0.07 ± 0.81	0.05	0.96 ± 0.90	–0.27 ± 0.88	0.05
Foot angle	–0.91 ± 0.89	0.10	1.06 ± 0.87	0.11	–1.52 ± 0.96	1.61 ± 0.93	0.11
Rear udder height	0.69 ± 0.74	0.08	0.22 ± 0.72	0.07	0.71 ± 0.80	–0.04 ± 0.77	0.08
Suspensory ligament	0.60 ± 0.88	0.16	0.47 ± 0.86	0.16	0.48 ± 0.95	0.30 ± 0.92	0.16
Teat placement	–0.29 ± 0.72	0.16	0.71 ± 0.70	0.16	–0.65 ± 0.78	0.95 ± 0.76	0.16
Fore udder attachment	0.33 ± 0.74	0.09	0.58 ± 0.72	0.09	0.13 ± 0.80	0.53 ± 0.77	0.09
Udder depth	0.53 ± 0.68	0.05	0.72 ± 0.66	0.05	0.30 ± 0.73	0.61 ± 0.71	0.05
Teat length	0.59 ± 0.70	0.12	–0.33 ± 0.68	0.12	0.84 ± 0.75	–0.63 ± 0.73	0.12

[1]α as defined in Falconer and MacKay (1996).
[2]DRBV = De-regressed breeding values.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

productive life was negatively correlated with fat yield ($P < 0.001$), fat percent ($P < 0.01$), and SCS ($P < 0.001$). Although these correlations were significant, or even highly significant in some cases, they were all very small.

DISCUSSION

In order to elucidate a residual QTL effect on proximal BTA14, having been discussed in recent publications (Bennewitz et al., 2004; Fisher and Spelman, 2004), attention was focused on the CYP11B1 gene within the QTL region, which influences energy metabolism. By designing selective PCR primer systems, it was possible to amplify, sequence, and genotype a polymorphism (V30A) in exon 1 of the coding bovine CYP11B1 gene with reliability. Although this gene had found scarce mention in genetic mapping as a molecular marker on BTA14, there was no indication of how interference with the CYP11B1 pseudogene in its close vicinity had been avoided.

Molecular Aspects

There are indications of a gene duplication event of the CYP11B1 ancestral gene before the mammalian radiation (Bülow and Bernhardt, 2002), leading to different sets of CYP11B1 genes in different species, not all of which retained functionality. Some apparently have an altered substrate range of action. Rats are known to have 3 (Mellon et al., 1995); humans are known to have 2 (Zhang and Miller, 1996); and cattle, sheep, and pigs are known to harbor only 1 functional CYP11B1 gene (Kirita et al., 1990; Okamoto et al., 1995; Sun et al., 1995; Bülow et al., 1996; Boon et al., 1997; Muller, 1998). Nonfunctional CYP11B1 genes (Kirita et al., 1990; Bülow and Bernhardt, 2002), resulting from an initial duplication or a secondary duplication event,

Table 6. Correlation between de-regressed breeding values (DRBV) of milk production traits, somatic cell score, fertility traits, and length of productive life for German Holstein cattle

Trait DRBV	Milk yield	Fat yield	Protein yield	Fat content	Protein content	Somatic cell score
Paternal calving ease	–0.07*	–0.10***	–0.06*	–0.02	0.05	–0.09**
Paternal stillbirth	0.05	0.02	0.08**	–0.02	0.04	–0.08**
Maternal calving ease	0.03	0.03	0.03	–0.01	–0.01	–0.02
Maternal stillbirth	–0.03	–0.03	–0.03	0.00	0.01	–0.03
Paternal nonreturn rate 90	0.05	0.02	0.05	–0.04	–0.02	–0.01
Maternal nonreturn rate 90	–0.06*	–0.12***	–0.09***	–0.06*	–0.04	0.02
Length of productive life	–0.03	–0.12***	–0.03	–0.08**	0.01	–0.52***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

have been found in many species, including cattle. Because of this, PCR amplification can be hampered by mixed amplifications from more than the targeted gene. Therefore, it is crucial to develop primer systems that selectively amplify only the coding gene. This is not easily accomplished because sequence similarities between the actual gene and the pseudogene can impair selectivity. In this study a deletion of 4 nucleotides in the 5′-region of the pseudogene compared with the coding gene presented the opportunity to amplify selectively from the coding CYP11B1 gene genomic DNA, which was confirmed by DNA sequencing and single strand conformation polymorphisms.

Allelic Frequencies of CYP11B1^V and DGAT1^K

The estimated frequency of maternally descended CYP11B1^V alleles in German Holsteins was 0.78. An additional typing of 202 German Simmental (Fleckvieh) cattle showed a frequency of 0.73. Nearly identical frequency values of CYP11B1^V in 2 distinct breeds could point to a physiological basis. As the gene product of CYP11B1 acts as a bipotent enzyme in cattle, sheep, and pigs (Bülow and Bernhardt, 2002), both alleles (CYP11B1^V, CYP11B1^A) are involved in the interconversion of biologically active cortisol to inactive cortisone and vice versa, catalyzing reactions at positions 11 and 18 of the steroid molecule.

In recent publications DGAT1^K allelic frequencies in Holstein cattle ranged from 0.30 (Bovenhuis and Schrooten, 2002) through 0.35 (Winter et al., 2002), and from 0.42 (Kaupe et al., 2004b) to 0.63 (Grisart et al., 2002). Spelman et al. (2002) found frequencies of 0.24 to 0.71 depending on degree of influence of US/Canadian or Dutch sires. In the current study the frequency of maternally descended DGAT1^K alleles in sons of the German Holstein granddaughter-design equaled 0.549, a value almost identical to the 0.548 reported by Thaller et al. (2003), despite differences in family numbers and total population size.

Individual Effects of CYP11B1^V and DGAT1^K on Milk Production Traits

In order to attain comparability between previously published results (Thaller et al., 2003; Bennewitz et al., 2004) and results presented in this publication, despite differences in family numbers and population size, effects of CYP11B1^V and DGAT1^K on milk production traits were first estimated separately in fixed models, well aware that effects of linkage could not be differentiated between genes in this way. Results of Thaller et al. (2003) and Bennewitz et al. (2004) were based on daughter yield deviations (**DYD**) as dependent variables. For the current study, DYD were not available for all traits under consideration; therefore, DRBV were utilized (Table 2). Using DRBV is a recommended alternative where DYD are missing (Thomsen et al., 2001). The DYD by definition represent only half of the breeding value of sires, whereas de-regressed proofs represent complete breeding values; therefore, standard deviations of DYD are likewise only half the standard deviations of de-regressed proofs. Taking into account that estimated $\alpha/2$ effects of recent publications are half those presented herein, very similar values for DGAT1 effects on milk production traits were found.

Comparing results on the basis of DYD with the above-mentioned publications, DGAT1^K $\alpha/2$ for milk yield differed from 2 to 9 kg, and $\alpha/2$ for fat content and protein content were nearly identical. The $\alpha/2$ mean value for fat yield differed by 0.51 to 1.18 kg, and $\alpha/2$ for protein yield differed from 0.27 to 0.22 kg. Effects of all 5 milk production traits were highly significant for DGAT1^K as well as for CYP11B1^V, as could be expected because of linkage effects. The CYP11B1^V was significant for SCS, which was attributed to possible linkage to a QTL (ILSTS011-BM302) further away on the chromosome (Zhang et al., 1998).

Joint Effects of CYP11B1^V and DGAT1^K on Milk Production Traits

In order to distinguish CYP11B1^V from DGAT1^K substitution effects, both alleles were included as independent variables in 1 fixed model. As a result, substitution effects of each allele, as well as respective significances for milk production, fertility, and conformational traits, clearly changed values. The substitution effect for 1 CYP11B1^V allele showed milk yield to decrease, together with a highly significant increase in fat content but led to no change in fat yield because milk volume and fat yield or protein yield are closely correlated. For every substituted CYP11B1^V allele, protein content showed significant increase but in total led to significant reduction in protein yield because milk yield was reduced as well. Whereas CYP11B1^V had significant effects on SCS in the individual analysis, no significant effects were found in the joint analysis.

Bennewitz et al. (2004) revisited the BTA14 QTL, in order to test for additional variation in DGAT1^K substitution effects. A further conditional QTL showed a highly significant effect for fat and protein yield and content, but no effect for milk yield. As results in the present publication confirm only additional positive effects in fat and protein content, further sources of variation for milk characteristics would be expected in the BTA14 QTL-region.

Individual and Joint Effects of CYP11B1^V and DGAT1^K on Conformation

Estimation of effects on conformational traits (Table 5), separately for each allele, produced no significant effects for CYP11B1^V. Spelman et al. (2002) typed New Zealand Holsteins for effects of DGAT1 on conformational traits but found no significant effects. In the current study, German Holsteins showed high significance for strength and significant response for rump width

with respect to DGAT1^K, when typed individually. In the joint analysis CYP11B1^V again was without effect, but DGAT1^K retained significance for strength and rump width, suggesting differences in body build between German Holsteins of the 2 DGAT1 genotypes. In recent publications of genome wide QTL mapping, no conformation effects were reported for BTA14. However, marker density was lacking (Schrooten et al., 2000), with large interval size (Hiendleder et al., 2003) and low information content (Boichard et al., 2003) on proximal BTA14.

Individual and Joint Effects of CYP11B1^V and DGAT1^K on Fertility

Paternal calving ease was significantly affected by CYP11B1^V in the separate, as in the joint model, where the effect was intensified (Table 4).

Because the breeding value of paternal calving ease describes the tendency of calves from a particular sire to be born more easily (or with more difficulty) than an average calf, the significant positive effect of CYP11B1^V might be attributable to variation of growth dynamics in the unborn calf. However, this hypothesis remains to be tested, and in light of very low heritabilities of fertility traits and the significant effects of many nongenetic factors, attributing such effects to specific alleles could be misleading.

The DGAT1^K allele showed a significant negative effect on maternal nonreturn rate. Because follicular dynamics are altered by negative energy balance (Lucy et al., 1992), potential exists for variation in maternal nonreturn rate through variation in DGAT1^K frequencies. As a consequence of higher energy expenditure of the cow to produce more milk fat, the ovulation rate could be reduced.

Correlations Among Production, Health, and Fertility Traits

The most important culling reasons of Holstein dairy cattle in Germany in 2002 were fertility problems (20.6%), lack of udder health (15.2%), and leg problems (9.1%) (Bünger et al., 2002). The relative breeding value (BV) for SCS was most closely correlated to BV for length of productive life (0.42), followed by the combined BV for feet and legs (0.33). To validate putative effects of a high-energy expenditure of the cow for an increased fat yield on fertility and udder health, Pearson's correlation coefficients were calculated between DRBV of production traits, fertility traits, and SCS, as shown in Table 6. Functional and fertility traits have rather low heritabilities, generally leading to limited reliabilities for young sires. Holstein bulls considered in this analysis were born between 1986 and 1993 so that reliabilities were assumed to be of reasonable strength in order to attain plausible results (Table 2). This level of average reliabilities of bull proofs indicates an elevated number of daughters used for genetic evaluation.

Estimated correlations between de-regressed proofs were rather weak except for one (between SCS and length of productive life) but significant in some cases, which is probably attributable to the number of sires. Correlations between DRBV for fat percent and all fertility traits under consideration tended to be negative, even if values were not pronounced. Among others, the strongest negative correlation was found between DRBV for fat yield/protein yield and maternal nonreturn rate, with DRBV for milk yield showing significance as well. The DRBV for fat yield likewise was negatively correlated with paternal calving ease. Gregory et al. (1979) found reduced paternal calving ease to be a disadvantage in crosses with *Bos indicus* cattle, which are known as high milk fat producing cattle and are homozygous for DGAT1^K (Kaupe et al., 2004b).

Negative correlations of DRBV for length of productive life with fat yield, fat content, and SCS were among the strongest found in this survey. A high correlation between length of productive life and SCS, as found in this study, seems to confirm results of Nash et al. (2000), who found daughters of sires that transmit longer productive life having fewer and less severe clinical mastitis incidences. Because the extent of negative energy balance and the rate of recovery from energy imbalance appear to be important factors in the animal (De Vries and Veerkamp, 2000; Roche et al., 2000; Westwood et al., 2002), the possibility of a negative impact on reproductive traits through rising fat yield, and thereby on length of productive life of milking cows, seems plausible.

IMPLICATIONS

Taking CYP11B1 and DGAT1 effects into account when analyzing genetic variation of the BTA14 quantitative trait loci for milk production traits in a joint analysis, only slightly more genetic variation could be explained than with DGAT1 alone. Whereas coefficients of determination remained unchanged for fat yield, fat content, and protein content, additional effects on milk yield could be discerned. Evidence was found for a negative impact of higher energy expenditure in the cow for milk fat production on fertility, and in this way, on length of productive life. Among conformational traits, effects were observed for DGAT1^K.

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