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Mapping of Serum Amylase-1 and Quantitative Trait Loci for Milk Production Traits to Cattle Chromosome 4

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ABSTRACT

The present study was undertaken to confirm and refine the mapping of a quantitative trait locus in cattle for milk fat percentage that had earlier been reported to be linked to the serum amylase-1 locus, AM1. Five half-sib families from the previous study and 7 new ones were genotyped for nine microsatellite markers spanning chromosome 4. AM1 was mapped between the microsatellite markers BMS648 and BR6303. In a granddaughter design, interval mapping based on multiple-marker regression was utilized for an analysis of five milk production traits: milk yield, fat percentage and yield, and protein percentage and yield. In the families reported on previously, significant effects for fat and protein percentages were detected. In the new families, an effect on milk and fat yields was found. The most likely positions of the quantitative trait locus in both groups of families were in the same area of chromosome 4 in the vicinity of the obese locus. Direct effects of the obese locus were tested for using polymorphism in two closely linked microsatellites located 2.5 and 3.6 top downstream of the coding sequence. No firm evidence was found for an association between the obese locus and the tested traits.

(**Key words**: dairy cattle, interval mapping, quantitative trait locus, amylase-1)

Abbreviation key: **PCR** = polymerase chain reaction, **QTL** = quantitative trait locus, **SRB** = Swedish Red and White breed.

INTRODUCTION

Milk production traits are typical quantitative characteristics that are controlled by numerous genes and environmental factors. The partitioning of these traits into their underlying genetic constituents could have important economical implications if this information could be successfully incorporated into programs using marker-assisted selection (30). Also, it should be of fundamental biological interest to clone some of the genes that have a major impact on quantitative characters. Paterson et al. (24) and Lander and Botstein (16) introduced interval mapping as a means of detecting quantitative trait loci (**QTL**). More recently, their methods were extended to exploit the prevailing breeding structure in cattle, which is characterized by the use of progeny testing and the availability of large paternal half-sib families (11, 15). The successful use of interval mapping relies on the availability of detailed genomic maps. In the past few years, several relatively dense genetic maps of cattle have been produced (4, 5, 11, 20), which now permit the scanning of the genome in the search for QTL.

In a previous report, using a single-marker approach, Andersson-Eklund and Rendel (1) reported linkage of the serum amylase-1 locus, AM1, to a QTL affecting milk fat percentage in the Swedish Red and White breed (**SRB**). Those researchers investigated 14 paternal half-sib families that were all heterozygous at the AM1 locus. In 7 of those families, segregation of this potential QTL could be detected; the mean difference for sons receiving either of the two AM1 alleles varied between 3.2 and 6.3 units of EBV for fat percentage. Different families showed different linkage phases with the QTL.

The objective of the present study was to confirm and refine the mapping of this QTL using the multiple-marker regression method described by Knott et al. (15). The material included 5 of the

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previous families and 7 new families of the same breed. The cattle homologue of the human α1A-amylase locus, AMY1, has been assigned to bovine chromosome 3 using somatic cell hybrid mapping (34). Initial screening excluded the linkage between AM1 and microsatellites on chromosome 3 in the families of our study (M. Lindersson, 1997, unpublished data). An amylase locus in pigs was recently mapped to pig chromosome 18 (25). Comparative mapping data (6, 9) suggested cattle chromosome 4 as a likely candidate for the cattle homologue of this amylase. This article reports the assignment of AM1 and the linked QTL to cattle chromosome 4. Because the putative QTL was located in the vicinity of the obese locus, a potential candidate gene for milk fat traits, effects of this locus were also tested by using two microsatellites that were tightly linked to the gene.

TABLE 1. Breed and number of sons per grandsire.

Grandsire	Breed[1]	Sons
1[2]	SRB	43
2[2]	SRB	31
3[2]	SRB	36
4[2]	SRB	52
5[2]	SRB	33
6	SRB	51
7	SRB	42
8	SRB	30
9	SRB	45
10	SRB	36
11	SRB	34
12	SRB	42
13[3]	SLB	40
Total		515

[1]SRB = Swedish Red and White breed; SLB = Swedish Friesian breed.

[2]Families included in the previous study (1).

[3]Included only in construction of the linkage map.

MATERIALS AND METHODS

Families

Of the 7 SRB families that were considered to be heterozygous for the locus affecting milk fat percentage (1), only 5 could be retrieved in this follow-up study; for the two remaining families, insufficient semen samples were available for DNA extraction. Some sons also were missing for the accessible families. Discarded semen samples could potentially decrease the prospect of detecting QTL by introducing selection bias because semen from low merit individuals is more often discarded (11, 21). In order to confirm the previously found QTL in an independent sample, 7 additional half-sib families were included in the study. In addition, one family from the Swedish Friesian breed was genotyped but was only used for constructing the linkage map. The families were used in a granddaughter design according to the description by Weller et al. (36). A semen sample was available from a total of 415 sons. In addition, 100 sons from these families for which only the AM1 genotype was known were included in the analysis. Altogether 195 sons were in the families from the previous study, and 320 were from the new families. The number of sons per family varied between 30 and 52 (Table 1).

Genotyping

Seven microsatellite markers spanning chromosome 4 were chosen from published linkage maps (4, 5) and marker data reports (29, 32). In addition, two microsatellites, BM1500 and BM1501, located approximately 2.5 and 3.6 kb downstream from the stop codon of the obese locus, were used (33). The BM1500 primers were as reported, but the BM1501 primers were modified to 5′-TGCAATATCTT-GTCCTTCTTGC-3′ and 5′-ACAGGGCGTAGCAGT-ACAGG-3′ (R. T. Stone, 1996 personal communication). Lysed samples were prepared, and microsatellite loci were amplified separately in 10-μl reactions containing 1.5 m*M* $MgCl_2$, 200 μ*M* dNTP, 0.25 U of *Taq* polymerase (Promega Corp., Madison, WI), 1× polymerase chain reaction (**PCR**) buffer (Promega), and 2 pmol of each primer. For BR6303 and BM1501, 20 pmol of each primer were used. One primer in each primer pair was fluorescently labeled. The PCR were carried out for 35 cycles; denaturation was at 88°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 20 s. The first cycle used a denaturation temperature of 95°C for 2.5 min, and the following three cycles used a denaturation temperature of 94°C for 1 min. The PCR products from one to three microsatellites were separated in one lane on a 0.5-mm, 6% polyacrylamide gel, using an automated fluorescent DNA sequencer (A.L.F.™, Pharmacia, Uppsala, Sweden). A three-allele coding system was used for scoring genotypes (11). Alleles 1 and 2 denote the paternal alleles, and allele 3 represents all maternal alleles other than 1 and 2. For the microsatellites BM1500 and BM1501, the actual lengths of the alleles were determined using fragments of 115 and 195 bp from the κ-CN and β-LG genes, respectively, as standards (18). Typing of AM1 variants was done using a starch gel electrophoresis procedure essentially as outlined by Ashton (2).

Linkage Map Construction

Two-point and multipoint linkage analyses were performed using the CRIMAP Program Version 2.4 (12). The map was constructed using the BUILD option, and the FLIPS option was used to determine the reliability of the obtained order of markers. The CHROMPIC option was used to identify potential typing errors revealed as unlikely recombination events, which were reexamined. The recombination fractions, as reported in the BUILD output, were converted to map distances using the Haldane mapping function and were further used in the QTL analysis.

Interval Mapping

A regression method using multiple markers was adopted for the QTL analysis essentially as described by Knott et al. (15) and was applied to granddaughter designs as described by Spelman et al. (31) and Vilkki et al. (35). We assumed a model with one segregating QTL in the linkage group having an additive effect. The most likely chromosomal haplotypes of each grandsire were determined based on the observed marker genotypes of his sons in a way that minimized the number of recombination events between markers. The deduced haplotypes of each grandsire were arbitrarily designated 1 and 2. For each position, at 1-cM intervals, the probability of each son inheriting the first grandsire haplotype was calculated and was conditional upon the genotypes at the two nearest informative flanking markers (35). The EBV of the sons were used as data in the analysis for five milk production traits: milk yield, fat and protein percentages, and fat and protein yields, based on national evaluations (sire-maternal grandsire model). Trait scores were regressed onto the calculated probabilities of inheriting that particular DNA segment from the first grandsire haplotype. The following intraclass regression model was used for analyses at 1-cM intervals along chromosome 4:

$$Y_{ij} = \mu + a_i + b_i X_{ij} + e_{ij}$$

where

Y_{ij} = weighted EBV of son j of grandsire i,
μ = overall mean,
a_i = fixed effect of grandsire i,
b_i = regression coefficient within grandsire i,
X_{ij} = probability of son j receiving a putative QTL allele at a given position from the first haplotype of grandsire i, and
e_{ij} = residual random term.

The mean squares obtained from regression within grandsires were pooled, and the ratio of this regression to the pooled residual mean squares provided an F ratio used as a test statistic in the analysis across families. The F ratios can also be calculated for individual families in the analysis within families. The maximum value of this F ratio along the chromosome indicates the most likely position of the QTL.

The EBV for sons having few daughters are, to a larger extent, based on the information from relatives, which is generally undesirable in QTL analysis because the haplotypes of the relatives are unknown. The EBV were, therefore, weighted by their approximate reliability (i.e., the squared correlation between the true breeding value and the EBV). Weighting of the contribution from each son, however, should account for only minor effects in this study because the breeding values generally were based on large numbers of daughters, and the heritabilities for milk production traits are relatively high (0.25 for the yield traits and 0.5 for the percentage traits in this study).

We used a permutation test, as outlined by Churchill and Doerge (7), to determine the empirical significance thresholds that were used in the analysis. This method provides the means of obtaining appropriate thresholds, taking into account the precise characteristics of the respective trait and the experimental structure. Within families, the trait data (along with their weighting factors) were randomly shuffled, but the genotypes were retained. For each shuffle, the test statistic was calculated for each position, and this procedure was repeated 10,000 times. The highest value from each permutation test was picked and stored. These values were sorted, and the appropriate cutoff points were taken to provide the 0.1, 1, and 5% chromosomewise significance thresholds. Interval mapping methods involve the inherent problem of multiple testing, thus, demanding appropriate corrections of nominal significance levels (17). We chose not to adjust the significance levels for a total genome scan in this study because our intent was to follow up a narrow region of the genome that was earlier reported to harbor a potential QTL. The QTL analysis was performed separately for the groups consisting of the 5 former families and the 7 new families of the SRB breed.

Testing for a Direct Effect of the Obese Locus

The direct effects of haplotypes at the obese locus were analyzed in a multiple regression model in which the EBV of the bulls were regressed onto the

number of copies of each haplotype, which in a granddaughter design reflects the proportion of the daughters inheriting the haplotype. All 12 SRB families (Table 1) were analyzed together. Haplotype frequencies were calculated using the haplotypes transmitted from the dams to each son. Haplotypes with a frequency of less than 3% were pooled together to avoid extremely small classes. The following model was used:

$$Y_{ij} = \mu + a_i + \Sigma b_r X_{ijr} + e_{ij}$$

where

Y_{ij} = weighted EBV of son j of grandsire i,
μ = overall mean,
a_i = fixed effect of grandsire i (i = 1, 2 . . . 12),
Σb_r = summation of product of regression coefficients of bull EBV on the number of copies of each obese haplotype r (r = 1, 2 . . . 8), with x_{ijr},
X_{ijr} = 0, 1, 2, depending on the number of copies of obese haplotype r, and
e_{ij} = residual random term.

We imposed a constraint on the regression coefficients such that $\Sigma b_r = 0$ to avoid dependencies in the data, as suggested by Østergård et al. (23). Using this model, we tested for the substitution effect of each haplotype and for an overall effect of the obese locus. In the latter case, we performed an *F* test, taking the ratio of the pooled mean squares of the haplotypes divided by the mean square error. For the test of a direct effect of the obese locus, we used the general linear model procedure of SAS (28).

RESULTS

Linkage Map Construction

Paired two-point lodscore analysis showed significant linkage between AM1 and several microsatellites on chromosome 4 (Table 2). The male linkage map including all markers, with positions given in Haldane centimorgans, is displayed below the graphs shown in Figures 1 to 3. For each interval, the most likely order was found to be concordant with previously published maps (4, 5, 32). The map distances are also similar to those previously reported. We chose to use our own linkage map in the subsequent QTL analysis.

Interval Mapping

The QTL analysis for fat and protein percentages across the 5 families included in the previous study is shown in Figure 1, which also shows the chromosome significance levels. The support for a QTL on chromosome 4 was significant at 1% for both traits. Even if a Bonferoni correction was imposed, assuming three independent tests (31), the results were significant at 5%. The highest values for the test statistic profiles were at position 95 for both traits. The similar shape of both curves is not surprising, given the high correlation between the two traits. Both curves have a maximum at a position of one for the more informative markers (the AM1 locus), possibly reflecting a bias toward placing QTL at marker positions, despite the approach of taking all markers into account when the transmission probabilities are calculated. Spelman et al. (31) tested this possibility and found that, indeed, 70% of their QTL curves peaked at a marker point.

TABLE 2. Paired two-point linkage analysis of the serum amylase-1 locus AM1 against other marker loci on chromosome 4.

Loci	Recombination fraction	Lodscore
AM1-BM6458	0.25	4.0
AM1-OBS	0.16	8.6
AM1-BM5648	0.05	14.6
AM1-BR6303	0.10	20.1
AM1-MGTG4B	0.20	3.4

Examination of the five families individually revealed that the QTL effect on fat content was mainly

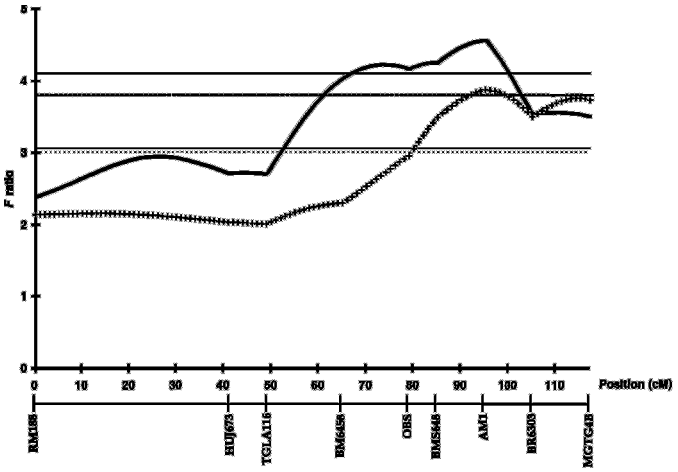

Figure 1. The *F* ratio curves for across-family analysis of fat percentage (thick solid curve) and protein percentage (crossed curve) in five families that were also part of an earlier study (1). The 1 and 5% chromosome thresholds for fat percentage are indicated by solid thick and solid thin lines, respectively. The 1 and 5% chromosome thresholds for protein percentage are indicated as dotted thick lines and dotted thin lines, respectively. Marker positions are indicated below the graph in Haldane centimorgans.

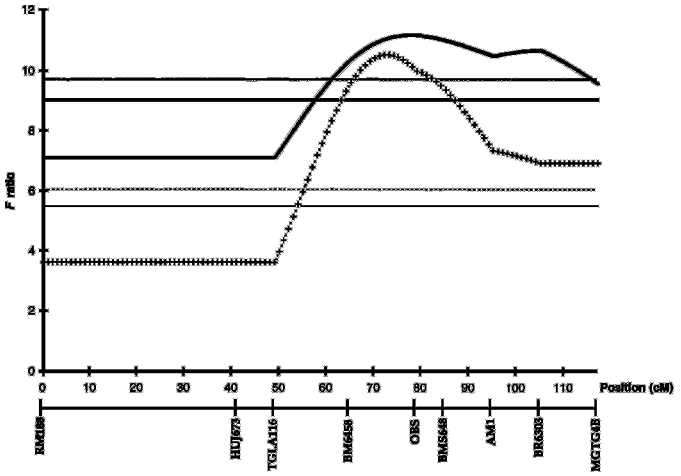

Figure 2. The curves for *F* ratio for within-family analyses of fat percentage in families 1 (crossed curve) and 3 (solid curve). The 1 and 5% chromosome thresholds for family 1 are indicated as dotted thick and dotted thin lines, respectively. The 1 and 5% chromosome thresholds for family 3 are indicated as solid thick and solid thin lines, respectively. Marker positions are indicated below the graph in Haldane centimorgans.

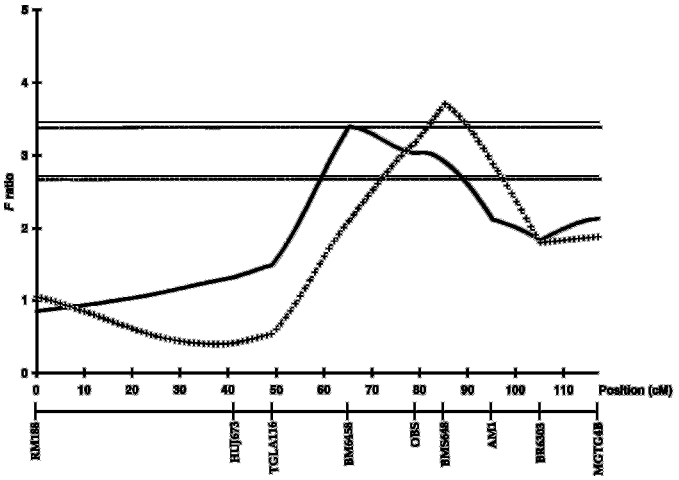

Figure 3. The *F* ratio curves for across-family analysis of milk yield (thick solid curve) and fat yield (crossed curve) in the new families. The 1 and 5% chromosome thresholds for milk yield are indicated as solid thick and solid thin lines, respectively. The 1 and 5% chromosome thresholds for fat yield are indicated as dotted thick and dotted thin lines, respectively. Marker positions are indicated below the graph in Haldane centimorgans.

due to significant effects in family 1 and 3. A plot of the within-family curves (Figure 2) showed that these two families have QTL curves of similar shape; maximum values occurred at 73 and 78 cM, respectively. This result demonstrates that the most likely position of the QTL might differ from position 95, which was indicated in the across-family analysis. However, the precision with which QTL could be mapped in the current study is rather poor because of the small family sizes. The results within families for protein percentage gave a different picture. In this case, QTL segregation was significant at the 5% level for families 1 and 2 but not for family 3. The contradictory results for families 2 and 3 regarding fat and protein percentages are reflected by a relatively lower correlation between the two traits within these two families (0.69 and 0.62, respectively) compared with the overall correlation (0.93) including all 13 families. The substitution effects (Table 3) for the QTL on fat content were 4.7 and 4.6 units of EBV for family 1 and family 3, respectively, which is approximately one standard deviation of the EBV or two-thirds of the phenotypic standard deviation of the trait. On an absolute scale, the effect corresponds to a difference of 0.2% in fat percentage between daughters inheriting the respective QTL allele.

The results from the sample comprising the 7 new families revealed no QTL effect on the fat and protein percentages. However, an effect ($P < 0.01$) on fat yield was found in the same area and was maximum at 85 cM (Figure 3). Also, an effect near the 1% level for milk yield was found; the peak position was 65 cM. Family 9 showed the largest effect for both characteristics. The most likely position for both traits within this family was at position 79, the position of the obese locus. The substitution effect within

TABLE 3. Substitution effects of quantitative trait loci in units of EBV, *F* ratios, and *P* values within individual families measured at the test statistic peak position.

Family	Trait	Peak position	*F* Ratio	*P*	QTL Effect	
					$\bar{X}$	SE
1	Fat content	73	10.5	0.007	4.7	1.4
3	Fat content	78	11.2	0.004	4.6	1.4
9	Fat yield	79	10.8	0.009	5.7	1.7
9	Milk yield	79	10.1	0.012	6.1	1.9

TABLE 4. Frequencies of different haplotypes of the two microsatellites BM1500 and BM1501 at the obese locus.

Haplotype	BM1500/ BM1501[1]	Frequency
1	135/164	0.038
2	135/168	0.003
3	135/172	0.069
4	135/174	0.276
5	135/176	0.310
6	135/180	0.003
7	143/161	0.052
8	143/164	0.165
9	143/166	0.014
10	143/174	0.007
11	145/164	0.056
12	145/166	0.007

[1]Allele lengths are given in basepairs.

this family was measured to 5.7 units of EBV for fat yield and 6.1 units of EBV for milk yield (Table 3).

Direct Effect of the Obese Locus

The bulls within the 12 SRB grandsire families were genotyped for the two microsatellites located 2.5 and 3.6 kb downstream of the obese gene (33). We detected three alleles at BM1500 and 8 alleles at BM1501 (Table 4). Twelve different haplotypes constituting these two microsatellites were recognized. The 7 most common haplotypes and a pool of the 5 remaining haplotypes were tested for association with milk production traits. No significant overall effect of the obese locus was observed. Not surprisingly, then, only weak evidence for effects of individual haplotypes was detected. Haplotype 5 had an effect on fat percentage ($P = 0.037$), and haplotype 3 had an effect on milk yield ($P = 0.034$). These individual effects may well represent type I errors, given the many haplotypes and traits tested.

DISCUSSION

In humans, the α-amylases constitute two groups of enzymes; the salivary variants denoted AMY1, and the pancreatic variants denoted AMY2. Both groups contain several isoenzymes, and pseudogenes are also present (13). The AMY1 and AMY2 genes form a gene cluster on human chromosome 1 (8). The cattle homologues of human AMY1 and AMY2 are placed on chromosome 3 in a region homologous to human chromosome 1 (19). Consequently, our AM1 locus does not represent a human homologue of the α-amylases. Three different isoenzymes of amylase have been detected in the serum of cattle (26), originally denoted AM1, AM2, and AM3. Biochemical characterization studies have suggested that AM1 might not represent a true α-amylase, but merely a maltase with α-amylase activity (3). Hence, Rozhkov et al. (27) proposed changing the names for AM2 and AM3 to AMY1 and AMY2, respectively, because these designations should represent the real α-amylases. We propose retaining the designation AM1 for the serum amylase locus mapped to cattle chromosome 4 in this study to avoid confusion with the AMY loci. No obvious homologue to the cattle AM1 locus has yet been assigned to the corresponding region in the human genome, chromosome 7 (Genome Data Base, April 1997). This mapping experiment illustrates the power of comparative genomics. After chromosome 3 was excluded, we were facing a total genome scan to find the chromosomal localization of AM1. However, we were guided toward cattle chromosome 4 by the recent mapping of a serum amylase locus to pig chromosome 18 (25). Pig chromosome 18 shares homology with a part of human chromosome 7 corresponding to cattle chromosome 4 (6, 9).

The QTL mapping for livestock species is at an early stage. The years to come will provide us with vast amounts of data, some of which could represent spurious linkages. An important task, therefore, will be to replicate, in independent samples, the results from earlier studies. The objective of this investigation was to map and confirm a QTL that had been reported earlier to affect milk fat content in the SRB breed (1) and to determine whether the effect was caused by a closely linked QTL with modest effect or a larger QTL that was more distantly positioned. By mapping the previously unassigned AM1 locus and thereby the linked QTL to chromosome 4, we have partly achieved this goal. The method for mapping in the present study and the relatively small sample size used make it difficult to determine more precisely the location of the QTL, although a position in the vicinity of the obese locus seems plausible, given the results in the two families having the largest effect.

Interestingly, the potential QTL that were identified in the new and former families are positioned in the same area of the chromosome, which suggests that they may actually represent the same QTL. The characteristics of the QTL are different, however. The QTL in the old families affects the fat and protein percentages, but the QTL in the new families mainly affects milk and fat yields. A biological explanation for such disparate effects is not easily implemented. A QTL affecting milk and fat yields, reported to be associated with the Weaver locus in the Brown Swiss breed, has earlier been identified (14). The Weaver

locus was subsequently mapped to chromosome 4 at a position 3 cM from the microsatellite TGLA116 (10), which was included also in this study. The possible QTL found in our new families has similar characteristics and could represent the same QTL, although the peak position in our material is approximately 30 cM from TGLA116. Georges et al. (11) conducted a genome scan in a search for QTL exploiting interval mapping and using 14 large half-sib families of the US Holstein breed. Those researchers detected linkage to putative QTL situated on five different chromosomes. However, none of those were on chromosome 4.

Positional cloning of individual QTL, at least using existing or near future techniques, will prove to be an immense task in livestock species. Nonetheless, QTL mapping efforts could point out chromosomal regions harboring genes that have large or medium effects. The wealth of information generated in other species (e.g., human and mouse) could then aid in selecting candidate loci in homologous regions that could be further characterized and analyzed in cattle using association studies. One such candidate locus tested for in this study was the obese locus, coding for the hormone leptin. Leptin has lately received considerable interest because of its effect on fat metabolism and the resulting amount of stored body fat (22). The cattle obese gene has been mapped to chromosome 4 (32) and is positioned 16 cM proximal to the AM1 locus (this study). Interestingly, we have observed differences in the amount of body fat deposited in two lines of cows that were selected for high or low milk fat percentages but that had equal total energy production in the milk (M. Åkerlind, April 1997, personal communication). This result suggests that milk fat percentage can be influenced by genes that affect body fat deposition, thus, providing a rationale for investigating the obese gene. The present investigation, however, provided no firm evidence for a direct effect of the obese locus on the breeding value of bulls for milk fat yield. We are currently investigating whether such an association can be detected in the two selection lines. In this study, we examined the obese locus using closely linked, polymorphic microsatellites. It would be worthwhile also to test for an association with polymorphic sites within coding and regulatory parts of the gene.

CONCLUSIONS

The AM1 locus in cattle, which has been previously reported to be linked to a QTL affecting milk fat percentage, was mapped to chromosome 4. In light of the results from a new set of paternal half-sib families, the association between AM1 and milk fat percentage however, is not indisputable, although results from the screening of this chromosome indicate the presence of a QTL affecting milk production traits. A candidate gene for milk fat yield that is located on chromosome 4 is the obese locus because of its effect on fat metabolism. However, no clear evidence for such an effect was found in the present material. The QTL for milk production traits on bovine chromosome 4 needs to be pursued in subsequent studies before definite conclusions can be drawn regarding the precise location and nature of this QTL.

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