Refined positioning of a quantitative trait locus affecting somatic cell score on chromosome 18 in the German Holstein using linkage disequilibrium

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ABSTRACT

Combined linkage and linkage disequilibrium analysis (LALD) was conducted to more accurately map a previously reported quantitative trait locus (QTL) affecting somatic cell score on bovine chromosome 18. A granddaughter design consisting of 6 German Holstein grandsire families with 1,054 progeny-tested genotyped sons was used in this study. Twenty microsatellite markers, 5 single nucleotide polymorphisms, and an erythrocyte antigen marker with an average marker spacing of 1.95 cM were analyzed along a chromosomal segment of 50.80 cM. Variance components were estimated and restricted maximum likelihood test statistics were calculated at the midpoint of each marker interval. The test statistics calculated in single-QTL linkage analysis exceeded the genome-wide significance threshold at several putative QTL positions. Using LALD, we were successful in assigning a genome-wide significant QTL to a confidence interval of 10.8 cM between the markers ILSTS002 and BMS833. The QTL in this marker interval was estimated to be responsible for between 5.89 and 13.86% of the genetic variation in somatic cell score. In contrast to the single-QTL linkage analysis model, LALD analyses with a 2-QTL model confirmed the position of one QTL, but gave no conclusive evidence for the existence or position of a second QTL. Ultimately, the QTL position was narrowed down considerably compared with previous results with a refined confidence interval of less than 11 cM.

Key words: mastitis, somatic cell score, linkage disequilibrium, *Bos taurus* autosome 18

INTRODUCTION

Mastitis is one of the most persistent and expensive problems in the dairy production industry (Seegers et al., 2003). Somatic cell score is commonly used as an in-

dicator trait for mastitis and is implemented in routine sire evaluation in many countries (Rupp and Boichard, 2003). Although management practices play the most important role in mastitis prevention, heritability for SCS has been estimated to be as high as 16 to 17% (e.g., Liu et al., 2004). Because of the unfavorable correlation between SCS and milk production traits (Rupp and Boichard, 2003), SCS has proven difficult to improve genetically. The opportunity to address shortcomings in conventional selection for SCS, low selection intensity, and the low reliability of EBV for SCS at an early age makes this trait a prime candidate for use in marker-assisted selection (MAS) programs.

Several whole-genome scans have been conducted in the search for QTL underlying the genetic variance of SCS. Over 50 independent regions have been found in various populations on almost all bovine chromosomes (for review, see Khatkar et al., 2004). The telomeric end of *Bos taurus* autosome (BTA) 18 has been repeatedly identified as a likely region for harboring significant SCS QTL in various populations, including the United States Holstein (Ashwell et al. 1997; Rodriguez-Zas et al., 2002), Finnish Ayrshire (Schulman et al., 2004; Lund et al., 2007), Swedish Red and White (Holmberg and Andersson-Eklund, 2004; Lund et al., 2007), Swedish Holstein (Holmberg and Andersson-Eklund, 2004), Danish Red (Lund et al., 2007), and German Holstein (Brink, 2003; Kühn et al., 2003; Xu et al., 2006). Despite these efforts to pinpoint the specific chromosomal region affecting phenotypic variation in SCS, the causal mutation has not yet been identified. The existence of more than 1 QTL affecting SCS in the telomeric part of BTA18 has also been suggested (Brink, 2003; Xu et al., 2006).

The efficient implementation of MAS requires identification of marker(s) tightly linked to QTL, or discovery of the specific causative mutation. An initial family-based MAS pilot project proved the usefulness of existing marker information regarding selection on the QTL for SCS on BTA18 in the German Holstein population (Kühn et al., 2008). Nevertheless, there is a need to narrow down the QTL position on BTA18 to increase the efficiency of MAS and to provide a reasonable basis

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Figure 1. Marker map for the middle to telomeric region of *Bos taurus* autosome 18, including marker number, marker name, marker position (cM), absolute marker position (cM), number of alleles, number of informative meioses used for calculating the marker map, and the interval number, which corresponds to the putative QTL positions.

for the search for causal mutations. The purpose of this study was to further investigate the chromosomal regions affecting udder health on BTA18 in the German Holstein population. Marker density was considerably increased in comparison with previous studies (Brink, 2003; Kühn et al., 2003; Xu et al., 2006), and statistical models for single and 2-QTL analysis incorporating linkage disequilibrium methods were used to improve localization of the QTL within the context of a national research effort initiated by the German Förderverein Biotechnologieforschung.

MATERIALS AND METHODS

Families

Six paternal half-sib families comprising 1,054 genotyped bulls were analyzed. These animals are a subset of the German Holstein population and originate from the granddaughter designs used in the studies of Kühn et al. (2003) and Xu et al. (2006), which were conducted as part of the national QTL mapping project of German breeding organizations, several German animal breeding institutes, and animal computing centers. The number of bulls in each family ranged from 60 to 353, with an average of 175 bulls per family. A full pedigree including nongenotyped ancestors of genotyped animals was also available for estimating random polygenic effects (7,627 animals). Pedigree information was provided by the German genetic evaluation center (VIT) in Verden, Germany.

Phenotypes

The VIT provided phenotype information for genotyped animals, with daughter yield deviation (DYD) used as the phenotypic unit of measurement for SCS. The DYD for each bull were calculated as the average of the daughters' performance adjusted for the fixed and nonrandom effects of the daughters and the genetic effects of the bull's mates following Liu et al. (2004). Compared with EBV, DYD provide a more independent measure of phenotypic performance, because DYD describe the genetic value of the sires more accurately due to the adjustment of the daughters' dam (Bennewitz et al., 2004). The DYD were obtained from the official release of the April 2008 genetic evaluation and were calculated based on a random regression test-day model (Liu et al., 2004). The DYD for the first lactation were analyzed, as the number of offspring was highest in this parity.

Reliabilities associated with the DYD used in this study were obtained from the official release of the January 2008 genetic evaluation and were expressed as

the number of effective daughter contributions following Liu et al. (2004).

Marker Set and Genotyping

The marker set (Figure 1) included 26 markers (15 newly selected microsatellite markers, 5 SNP, and 6 markers that were genotyped within previous QTL mapping studies, 1 of which is a erythrocyte antigen; Brink, 2003; Kühn et al., 2003; Xu et al., 2006). The 20 newly selected microsatellite and SNP markers were chosen based on the information of the putative QTL positions for SCS on BTA18 reported by Brink (2003), Kühn et al. (2003) and Xu et al. (2006). For the selection of microsatellite markers, the bovine linkage map of the USDA's Meat Animal Research Center (MARC) was used (<http://www.marc.usda.gov/genome/genome.html>). The DNA of all sires was isolated from sperm samples provided by German AI stations. Genotyping of the microsatellite markers was performed by PCR or multiplex-PCR with fluorescence-labeled primers and a subsequent fragment length analysis using the MegaBACE 1000 DNA Analysis System and MegaBACE Fragment Profiler Version 1.2 software (GE Healthcare, Munich, Germany). The SNP were genotyped by standard RFLP techniques (Seyfert and Kühn, 1994). Thus, a PCR specific for the SNP and its flanking region was established and after amplification and restriction digest, the fragments were analyzed by agarose gel electrophoresis. To achieve high data quality, all genotypes were recorded independently twice and checked for Mendelian inheritance and unlikely double recombinants. Suspicious genotypes were retyped. After a second control, sires with conflicting genotypes were excluded from the analysis for the given marker.

Linkage Map

To evaluate the most likely marker order on BTA18, information from published linkage maps (Ihara et al., 2004; Snelling et al., 2005), radiation-hybrid maps (Itoh et al., 2005), human and bovine sequence assemblies (NCBI Map Viewer; <http://www.ncbi.nlm.nih.gov/mapview/>) as well as our own linkage mapping results were used to define a refined marker order. The marker order in the current bovine sequence assembly Btau4 is in accordance with our marker order, whereas there are several discrepancies to the previous bovine sequence assembly, Btau3.1. The genetic linkage map was calculated based on our genotyping data using CRIMAP software (Green et al., 1990). For some markers (BMON117, DIK4672, SNP523; SNP391, SNP398; BM2078, DIK4569, DIK4943), a recombination rate of zero was calculated. A marker spacing of 0.1 cM was

used in these cases to alleviate technical difficulties arising in the calculation of transmitting probabilities; the correct marker order was ensured through sequence information mentioned above.

Transmission Probabilities

The most probable linkage phases of genotyped sires were calculated using all marker genotypes to determine paternally and maternally inherited haplotypes. Sire transmission probabilities were calculated at the midpoint of each marker interval for later determination of the gametic relationship matrices. Both transmission probabilities and marker allele frequencies were calculated using BIGMAP (Reinsch, 1999). The midpoint of each marker bracket was considered a putative QTL position, resulting in 25 putative QTL positions.

Linkage Analysis

A model containing a random polygenic effect and a random additive QTL effect (Xu and Atchley, 1995; Hoeschele et al., 1997) was applied to the data for linkage analysis:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{W}\mathbf{v} + \mathbf{e} \quad [1]$$

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{W}_1\mathbf{v}_1 + \mathbf{W}_2\mathbf{v}_2 + \mathbf{e}, \quad [2]$$

where $\mathbf{y}_{(m \times 1)}$ is a vector of phenotypic observations (DYD) for sires; $\mathbf{X}_{(m \times k)}$ is a design matrix; $\boldsymbol{\beta}_{(1 \times 1)}$ is a fixed mean common to all observations; $\mathbf{Z}_{(m \times n)}$ is an incidence matrix relating animals to phenotypes; $\mathbf{u}_{(n \times 1)}$ is a vector of random polygenic effects; $\mathbf{W}_{(m \times gam)}$ is an incidence matrix; $\mathbf{v}_{(gam \times 1)}$ is a vector of random QTL effects; and $\mathbf{e}_{(m \times 1)}$ is a residual vector. Subscripts in parenthesis of the vectors and matrices denote their dimensions; m is the number of animals in the sub-pedigree of genotyped animals, k equals 1 in our study for the common mean, n is the number of animals in the extended pedigree, and gam is the number of unique allelic effects.

Random effects $\mathbf{u}$, $\mathbf{v}$, and $\mathbf{e}$ and their covariances have an expected value of zero. Their distribution is assumed with the multivariate normal densities: $\mathbf{u} \sim N(0, \mathbf{A}_p \sigma_p^2)$, $\mathbf{v} \sim N(0, \mathbf{G}_q \sigma_q^2)$, and $\mathbf{e} \sim N(0, \mathbf{D} \sigma_e^2)$, where σ_p^2 , σ_q^2 , and σ_e^2 describe the polygenic variance, the additive variance of the QTL, and the residual variance, respectively; $\mathbf{A}_p$ is the additive genetic relationship matrix; $\mathbf{G}_q$ is the covariance matrix for the additive effects of the QTL conditional on marker haplotypes within a 10-cM window around a given position with

haplotypes sorted according to their generation (i.e., founder haplotypes followed by haplotypes with known origin); and the diagonal matrix $\mathbf{D}$ containing 1 over the effective daughter contributions (Liu et al., 2004).

The covariance matrices ($\mathbf{G}_q$) for the additive effects of the QTL conditional on marker haplotypes in all analyses were calculated using the condensing algorithm of Tuchscherer et al. (2004), which has an effect similar to that of haplotype clustering (e.g., Calus et al., 2008). Haplotype groups are clustered if their correlation is high (>0.97); such haplotypes are considered "non-unique." The dimension of the resulting condensed gametic relationship matrices are no longer twice the number of genotyped animals, but contain 1 row and column for every unique haplotype effect. This method prevents quasi-linear dependencies that can occur when nearly identical rows and columns in the QTL covariance matrices occur.

The QTL test performed in all analyses was a likelihood ratio test, where the REML of the full model was compared with the REML of the model missing the QTL effect. The restricted log-likelihood ratio test (RLRT) statistic $RLRT^{Pos} = -2[\ln(L_0) - \ln(L_1^{Pos})]$ was calculated for each putative QTL position ($Pos = 1$ to 25), whereby L_1^{Pos} is the REML of the full model (model 1) for position Pos , and L_0 is that of the same model without random gametic effects for a given QTL. The additive relationship matrix $\mathbf{A}_p$ was the same for both models and included all relevant animals in the pedigree (7,627 animals). All calculations were done with a combination of own programs (Baes and Reinsch, 2007) and the ASReml package (Gilmour et al., 2006) as described in Baes and Reinsch (2008).

Incorporating Linkage Disequilibrium with Variance Components

In addition to the linkage analysis (LA), a linkage disequilibrium (LD) analysis and a combined LA and LD analysis (LALD) were performed. Both analyses involving LD are based on the assumption that similar marker haplotypes are more likely to have identical QTL alleles (Meuwissen et al., 2002). Covariance between founder haplotypes (i.e., maternal haplotypes of bulls and maternal and paternal haplotypes of their sires) was modeled by deriving the probability that QTL alleles carried by different marker haplotypes are identical by descent (IBD) as described by Meuwissen et al. (2002). If a common ancestor occurs within the known pedigree, IBD probabilities are calculated from the marker information (i.e., as in LA). However, if no common ancestor exists (e.g., founder haplotypes), the probability of being IBD at the QTL is estimated from

the similarity between founder marker haplotypes. In this way, IBD submatrices containing the covariances between founder haplotypes were used to supplement the $\mathbf{G}_q$ matrix with LD information.

Identity by descent submatrices were estimated for every putative QTL position in our study. The IBD probabilities contained in these matrices were used to fill the rows and columns corresponding to the respective founder haplotypes in the subsequent $\mathbf{G}_q$ matrices. The IBD probability for a given haplotype combination was therefore considered equivalent to the covariance between the specific haplotypes.

In a simulation study with evenly spaced markers, Grapes et al. (2006) found that fitting a 4 to 6 marker haplotype as a sliding window across the region resulted in the greatest accuracy compared with that of the other haplotype sizes tested. For practical applications, however, it may be advantageous to define the size of the window surrounding the putative QTL in centimorgans instead of the number of markers (i.e., when markers are unevenly spaced). In this study a haplotype was defined as the marker combination within a 10-cM window (5 cM left and right of the putative QTL position), which equated to an average haplotype size of 6 markers and ensured that each window contained at least 1 marker on each side of the putative QTL. We tried other window sizes (5 and 20 cM); however, the results did not differ markedly in these analyses.

One IBD submatrix for every putative QTL position was formed, resulting in 25 different IBD submatrices. These matrices were tested for positive definiteness; matrices with negative eigenvalues were appropriately adjusted using the BENDPDF program (Ebl, 1991). Bending was applied to 8 of 25 IBD submatrices. The average bending factor was 0.077. The $\mathbf{G}_q$ matrix in model 1 was supplemented with the IBD submatrices containing the covariances between founder marker haplotypes with no known ancestors as described by Meuwissen et al. (2002). In this way, the LA model described in the previous section becomes a combined LALD model when the IBD submatrices are included and the $\mathbf{G}_q$ matrix becomes 100% dense. The LD analysis was carried out similarly to the combined analysis; however, information on marker transmission from parents to offspring was ignored by setting paternal transmitting probabilities to 0.5.

Two-QTL Model

A 2-QTL model was calculated using a similar model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{W}_1\mathbf{v}_1 + \mathbf{W}_2\mathbf{v}_2 + \mathbf{e}, \quad [2]$$

where $\mathbf{W}_{i(m \times gam)}$ is an incidence matrix and $\mathbf{v}_{i(gam \times 1)}$ is a vector of random QTL effects for the putative QTL i ($i = 1$ to 2). Dimensions and other effects are as in model [1]. The RLRT ($RLRT^{Pos} = -2[\ln(L_0) - \ln(L_1^{Pos})]$) was calculated for each pair of putative QTL positions.

Statistical Inference

Significance thresholds were calculated as RLRT equivalents of logarithm of odds (LOD) units, where a LOD score >2 indicates chromosome-wide significance and a LOD score >3 indicates genome-wide significance (Lander and Botstein, 1989). A LOD score >2 is equivalent to a RLRT value of 9.2, and a LOD score >3 is equivalent to a RLRT value of 13.8 (Ott, 1991, pp. 65). Likewise, the LOD drop-off method of Lander and Botstein (1989) was employed to estimate approximate confidence intervals, which can be calculated by identifying the highest point in the RLRT profile and subtracting 1 LOD unit (equivalent difference in RLRT = 4.6); all positions within 1 LOD unit from the maximum are considered within the confidence interval. Asymptotically, these are approximately equivalent to a 96% confidence interval (Mangin et al., 1994).

RESULTS

Markers and Maps

Marker spacing ranged from 0.10 to 7.9 cM, with an average marker spacing of 1.95 cM. The markers had an average of 8.19 alleles, ranging from 2 for SNP markers to 34 for the erythrocyte antigen marker EAC. Average marker heterozygosity was 0.59 across all markers (range of 0.19 to 0.91). The number of informative meioses for markers and the number of alleles per marker is shown in Figure 1. There was an average of 365 informative meioses per marker with a range from 0 to 707.

LA

The RLRT profile of LA conducted using a model containing a single QTL for SCS and random polygenic effects compared with a model containing only random polygenic effects is shown in Figure 2a). The profile is plotted for putative QTL located at the midpoint of every marker interval. Putative QTL positions within a 1-LOD drop-off interval are shown in Table 1. The global maximum was located in marker interval 4 at position 68.55 cM (RLRT = 19.53) between BMS2639 and DIK4960, followed closely by interval 5 between DIK4960 and BMON117 at position 69.75 cM (RLRT = 19.34).

Figure 2. Restricted log-likelihood ratio profiles for a) linkage analysis, b) linkage disequilibrium analysis, and c) combined linkage and linkage disequilibrium analysis for a putative QTL affecting SCS on *Bos taurus* autosome 18. The lower dotted line (....) indicates the chromosome-wide significance threshold, the upper dashed line (---) indicates the genome-wide significance threshold. The thick black line in (c) indicates an approximate 96.8% confidence interval.

LD and LALD Analysis

The analysis based on LD provided the likelihood profile shown in Figure 2b). One distinct peak between markers SNP523 and BMS833 at marker interval 8 can be observed, although the RLRT at this position does not reach the significance threshold (RLRT = 7.86). The likelihood profile of the combined LALD analysis (Figure 2c) is similar to that of the LA, although the maximum test statistics are slightly higher in the combined analysis. Maxima were located in marker interval 7 at position 70.55 cM (RLRT = 21.89) and interval 8

at position 72.55 cM (RLRT = 21.78). A 1-LOD drop-off interval was calculated, which encompasses marker intervals 3 to 8 (10.50 cM total length). Putative QTL positions within the 1-LOD drop-off interval are shown in Table 1. The ratio of QTL variance to total genetic variance was estimated between 5.89 and 13.86% of the additive genetic variation in SCS in the German Holstein in this region.

Two-QTL LALD and LD Analyses

A contour plot of the likelihood profile for an analysis using a 2-QTL LALD model is shown in Figure 3. The QTL located between markers DIK4672 and BMS833 is again obvious because of the clear “likelihood ridge” between positions 70.55 and 72.55 cM. This ridge is visible in Figure 3 as dark horizontal and nearly parallel contour lines. Test statistics for the marker intervals 7 and 8 exceed the genome-wide significance threshold for all possible putative second QTL positions. The highest likelihood was found for the QTL pair at intervals 7 and 25 (RLRT statistic = 23.51); however, the RLRT statistics at intervals 6 and 25 and intervals 8 and 3, 4, 6, 7, 13, and 25 were all comparably high (RLRT statistic ranged from 22.67 to 23.45) with no obvious position for a second QTL. Moreover, the difference between likelihoods in the single-QTL LALD model and the 2-QTL LALD model was too small (difference in RLRT of 1.62) to provide evidence for a 2-QTL hypothesis.

DISCUSSION

Previous studies used LA to investigate data for SCS on BTA18; this is, to the best of the authors' knowledge, the first study that applies LD and combined LALD methods to information on BTA18. By applying these methods in combination with an increased marker density, we were able to narrow the QTL region with effect on SCS previously identified by Brink (2003) and Xu et al. (2006) to a 10.5-cM interval. Xu et al. (2006) and Kühn et al. (2003) identified the telomeric end of BTA18 with marker TGLA227 as being linked to a QTL for SCS in an LA. However, in our LA, there was no longer a peak near TGLA227. In agreement with this, the 2-QTL LALD analysis showed no indication of a second QTL in the interval DIK5235–TGLA227 or elsewhere. In all previous studies testing for a linked QTL, TGLA227 was considered the last marker on the map; only 1 marker (DIK4013) is located more distally in the MARC USDA bovine linkage map. The inclusion of DIK4013 in our study could have had an effect on the significance of the LD analyses in the telomeric region of BTA18. Markers 5 cM left and right of the

Table 1. Marker intervals¹ exceeding the 1-logarithm of the odds drop-off threshold using linkage analysis (LA) and combined linkage and linkage disequilibrium analysis (LALD) for SCS QTL on *Bos taurus* autosome 18 in the German Holstein

Method	Interval	Position, cM	Marker 1	Marker 2	RLRT ²	Polygenic variance	QTL variance	QTL/genetic variance, %
LA	3	66.05	ILSTS002	BMS2639	17.734	0.1681	0.03071	15.45
	4	68.55	BMS2639	DIK4960	19.53	0.1661	0.03287	16.52
	5	69.75	DIK4960	BMON117	19.344	0.1624	0.03729	18.68
	6	70.45	BMON117	DIK4672	18.266	0.1669	0.03236	16.24
	7	70.55	DIK4672	SNP523	18.328	0.1665	0.03283	16.47
	8	72.6	SNP523	BMS833	18.14	0.1654	0.03374	16.94
	9	75.5	BMS833	DIK4232	16.044	0.1664	0.03332	16.68
	3	66.05	ILSTS002	BMS2639	19.788	0.1807	0.02415	11.79
	4	68.55	BMS2639	DIK4960	18.888	0.1892	0.01185	5.89
LALD	5	69.75	DIK4960	BMON117	20.184	0.1878	0.01223	6.11
	6	70.45	BMON117	DIK4672	21.616	0.1851	0.01318	6.65
	7	70.55	DIK4672	SNP523	21.89	0.1850	0.01423	7.14
	8	72.6	SNP523	BMS833	21.784	0.1778	0.02860	13.86

¹Marker 1 = first marker of marker interval; marker 2 = second marker of marker interval.

²RLRT = restricted likelihood ratio test statistic.

putative QTL position were considered a haplotype in the LD analyses; therefore, the haplotype for interval 24 (between DIK5235 and TGLA227) included DIK4013. At the end of the chromosome, the window used for haplotype characterization is limited on one side and contains fewer markers than those for markers in the middle of the chromosome. It may be more difficult to detect linkage disequilibrium to a QTL at the borders of the investigated region. The use of SNP markers, which are much more frequent in the genome, will provide a good possibility for increasing marker density around TGLA227 to further investigate the telomeric region of BTA18.

Any comparison between our results and previously published studies is hampered by the lack of information on confidence intervals and limited marker densities in previous analyses. Ashwell et al. (1997) found a QTL (nominal $P \leq 0.01$) on BTA18 at the marker BM2078 using single marker regression across families in the US Holstein population, which is likely the same QTL reported in one family of the same population by Rodriguez-Zas et al. (2002). Although Muncie et al. (2006) also analyzed US Holsteins, they did not report this QTL, nor did they report any chromosome-wide significant regions within the region under investigation in the present study.

In a study by Schrooten et al. (2000) on Dutch Holsteins, 7 markers spanning 124 cM (average marker interval = 20.7 cM) on BTA18 were included in a whole-genome scan for conformation and functional traits. A QTL affecting SCC with a chromosome-wide $P \leq 0.01$ was found between BM7109 and ILSTS002. This chromosomal region was included in our study, but our results do not support the earlier findings. The marker map of Schrooten et al. (2000) is, however, difficult to

compare with our map because their calculations were based on only 7 markers; it is likely that marker positions are quite different from ours in terms of interval size and total chromosomal length.

In contrast to those in other countries, breeding programs for udder health traits in Denmark, Finland, Norway, and Sweden include clinical mastitis (CM) in addition to SCC/SCS. Clinical mastitis is a binary trait based on whether or not the cow had at least one veterinary treatment for CM shortly before or during the lactation (Klungland et al., 2001). In a study by Holmberg and Andersson-Eklund (2004) on Swedish Red and White and Swedish Holstein animals, QTL for both CM and SCC were found on BTA18; however, the QTL were not significant in an across-family analysis. Schulman et al. (2004) studied the Finnish Ayrshire population and found similar results: genome-wide significant QTL for both SCS and mastitis were localized near TGLA227. Lund et al. (2007) examined BTA18 in the search for CM and SCS QTL in Finnish Ayrshire, Swedish Red and White, and Danish Red animals using within-family (LA) variance component analysis. They did not detect QTL for CM; however, the RLRT test statistics for SCS and a 2-trait model with a pleiotropic QTL affecting both CM and SCS reached chromosome-wide significance over the entire telomeric end of the chromosome (35.1 cM). Seven of 8 markers (BM7109, ILSTS002, BMS2639, BMON117, BM2078, BM6507 and TGLA227) analyzed by Lund et al. (2007) were also included in our study.

Compared with the previously mentioned studies, we increased marker density and incorporated LD methods in single- and 2-QTL models to improve localization of the QTL affecting SCS repeatedly reported on BTA18. A large chromosomal region (>30 cM) was identified

using LA, whereby LD narrowed the test statistic profile down to a single peak at position 72.55 cM between the markers SNP523 and BMS833. Likelihood values were generally higher in the 2-QTL model than in the single QTL model (23.51 vs. 21.89), but no conclusion regarding further QTL affecting udder health could be drawn.

CONCLUSIONS

This study was undertaken with the goal of further investigating the chromosomal area affecting SCS on BTA18 identified by Kühn et al. (2003) and Xu et al. (2006) by using a much denser marker map and

employing LD methods. Compared with the point of departure, a region of interest for future fine-mapping experiments was narrowed to the marker interval between ILSTS002 and BMS833, with an approximate confidence interval spanning less than 11 cM. Although a 2-QTL model showed genome-wide significance for a putative QTL between DIK5235 and TGLA227 in connection with the putative QTL between DIK4672 and SNP523, no conclusive evidence for the existence or position of a second QTL could be found. Further studies regarding the QTL at the telomeric end of BTA18 are required, in which the use of densely spaced SNP near TGLA227 may provide a clearer picture of the linkage disequilibrium in this region.

Figure 3. Contour plot of the restricted log-likelihood ratio profile using combined linkage and linkage disequilibrium analysis for a 2-QTL model affecting SCS on *Bos taurus* autosome 18. Position (cM) is given on the x- and y-axes. The graphic can be considered topographical, with darker lines indicating a higher likelihood test statistic. A clear likelihood ridge is seen across position 70.55 to 72.55 cM. Results are mirrored across the diagonal (mirror image). RLRT = restricted likelihood ratio test statistic.

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