Session 35: Theatre Presentation Nr. 8: Correspondence: reinsch@fbn-dummerstorf.de

The Quest for Genetically Improved Udder Health: Fine Mapping a QTL for Somatic Cell Score in the German Holstein

Christine Baes[1], Manfred Mayer[1], Jens Tetens[2], Jörn Bennewitz[2], Zengting Liu[3], Friedrich Reinhardt[3], Georg Thaller[2], Norbert Reinsch[1]

[1]*Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, D-18196 Dummerstorf, Germany*
[2]*Institut für Tierzucht und Tierhaltung, Christian-Albrechts-Universität, D-24098 Kiel, Germany*
[3]*Vereinigte Informationssysteme Tierhaltung w.V., D-27283 Verden, Germany*

INTRODUCTION

Subclinical or clinical inflammation of the mammary gland is the principal manifestation of poor udder health in dairy cattle. Usually caused by microbial infection, mastitis remains one of the most expensive and persistent health problems in the dairy sector. Although management practices play the largest role in the fight against mastitis, some animals are more genetically disposed to infection than others. Genetic selection for udder health in Germany is achieved through the indicator trait somatic cell score (SCS), which is highly correlated with clinical mastitis and has a moderate heritability. Through the use of genetic markers, it is possible to find quantitative trait loci (QTL) which underlie the genetic variance of udder health traits such as SCS. Incorporation of such QTL in breeding schemes via marker assisted selection (MAS) has the potential to improve udder health genetically and to achieve higher mastitis resistance without the use of antibiotics or vaccines.

A number of QTL affecting udder health traits have been reported (see KHATKAR ET AL. 2004 for a review), however linkage disequilibrium (LD) detected for these QTL was only found within individual families. Linkage (LE) and linkage disequilibrium within and between families can be exploited to fine map QTL using combined LD/LE analysis (MEUWISSEN AND GODDARD (2001). If linkage disequilibrium presides on a population level (i.e. between families), specific marker haplotypes may be identified which have predictive ability over the entire population.

The aim of this study was to use an LE analysis, an LD analysis and a combined LD/LE analysis based on variance component estimation to fine map QTL affecting udder health on BTA27 previously reported in the German Holstein population. The combined LD/LE analysis models expected covariances between haplotype effects, which are proportional to linkage disequilibrium in the population at a given QTL position. A further goal of this analysis was to identify specific haplotypes associated with increases or decreases in mastitis resistance in this population.

MATERIALS AND METHODS

Genetic Material and Phenotypic Measurements

Marker information was obtained from the genome analysis project of the Federation of German Cattle Breeders (ADR) and is currently used for the marker assisted BLUP evaluation of the somatic

cell score trait. The pedigree and phenotype information were obtained from the genetical computing centre (VIT) in Verden, Germany.

Six paternal half sib German Holstein families with a total of 492 bulls genotyped for 19 markers were included in this study. A pedigree containing 4,622 German Holsteins, including non-genotyped ancestors of genotyped animals, was also available. The number of genotyped sons per sire ranged from 28 to 230, with an average family size of 82 sons per sire.

Phenotype information for genotyped offspring was available in the form of daughter yield deviations (DYD) from the routine German Holstein genetic evaluation. The DYD were weighted using the number of effective daughter contributions (Liu et al. 2004). Daughter yield deviations ranged from -0.129 to 0.102 (average = -0.0132), with lower values representing a lower somatic cell count and therefore favourable effects on udder health.

Genotypes and Linkage Map Construction

Eighteen microsatellite markers were chosen from the MARC USDA bovine linkage map (http://www.marc.usda.gov/genome/genome.html). Additionally, one marker was developed within this study (data not shown) resulting in a total of 19 microsatellite markers used for analysis.

The average marker interval was 1.78 cM with a maximum of 12.7 cM and a minimum of 0.02 cM between the markers. There were an average of 6.84 alleles per marker with a maximum of 11 alleles and a minimum of 2 alleles. The midpoint of each marker interval was regarded as a putative QTL position (see Figure 1).

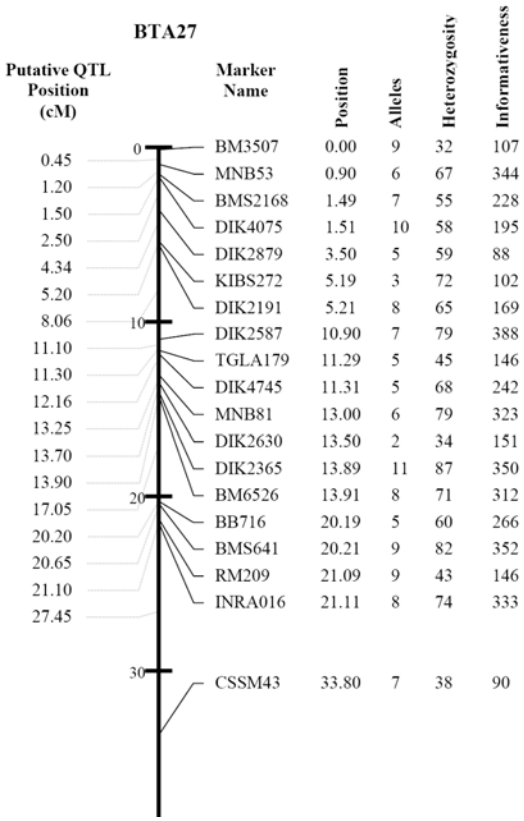

Figure 1. Marker and QTL map spanning over 33.8 cM. QTL positions are given to the right and marker positions to the left of the index. Midpoint of each marker interval is considered a new putative QTL position.

Statistical Analysis

Haplotypes of grandsires and sires, as well as marker allele frequencies, were determined using BIGMAP software (REINSCH, 1999). The condensed gametic relationship matrix (TUCHSCHERER ET AL. 2004) was computed at the midpoint of each marker bracket along the chromosome using COBRA (BAES AND REINSCH, 2007). A window of 10 cM (5cM on each side of the putative QTL position) was used, with all markers occurring within this window included in the analysis for the given position.

Correlations between founder haplotypes were assumed zero in the LE analysis. In the combined LD/LE analysis, the gametic relationship matrix was supplemented with IBD probabilities between founder haplotypes using the method described by MEUWISSEN AND GODDARD (2001). The LD analysis was carried out similarly to the LD/LE analysis, however transmitting probabilities between all non-founder haplotypes were set to 0.5.

The following mixed linear model was applied in ASReml (GILMOUR ET AL., 2006) to calculate the restricted maximum likelihood estimates of the variance components at each putative QTL position (gametic effects model):

$$\mathbf{y}_i = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{Wv} + \mathbf{e}$$

where $\mathbf{y}_{i(m\times1)}$ is the vector of phenotypic observations for sire *i*, $\boldsymbol{\beta}_{(k\times1)}$ is the vector of fixed effects, $\mathbf{u}_{(n\times1)}$ is a vector of random additive genetic effects due to loci not linked to the putative QTL, $\mathbf{v}_{(gam\times1)}$ is a vector of random allelic effects at the marked QTL and $\mathbf{e}$ contains random residual effects. Subscripts in parenthesis of the vectors and matrices denote their dimensions; *m* is the number of observations, *k* is an average equal to 1, *n* is the number of animals in the full pedigree and *gam* is the number of unique allelic effects. In this model, $\mathbf{v}$ represents the combined maternal and paternal haplotype effects as a single additive component. The model also contains the known incidence matrices $\mathbf{X}_{(m\times k)}$, $\mathbf{Z}_{(m\times n)}$ and $\mathbf{W}_{(m\times gam)}$. Expectations of $\mathbf{u}, \mathbf{v}$ and $\mathbf{e}$ and their covariances were assumed to be zero.

The restricted likelihoods were maximized at each putative QTL position. The asymptotic distribution of the restricted log likelihood ratio test (RLRT) statistic, $\mathrm{RLRT} = -2\ln(L_0 - L_P)$, was calculated, whereby L_P is the restricted log likelihood of the model shown above and L_0 is that of the same model without random allelic effects at a given QTL.

RESULTS AND DISCUSSION

The RLRT curves for the LD, LE and LD/LE are displayed in Figure 2. The results indicate distinct LE, LD and combined LD/LE peaks at 4.34 cM in marker interval 5. The peaks in interval 5 are located within a marker interval of 1.79 cM. At this position, 5 markers to the left of the QTL and 2 markers to the right of the QTL were used for calculation of the supplemental IBD probabilities. The number of genes in this interval can be roughly estimated at about 20, however more detailed

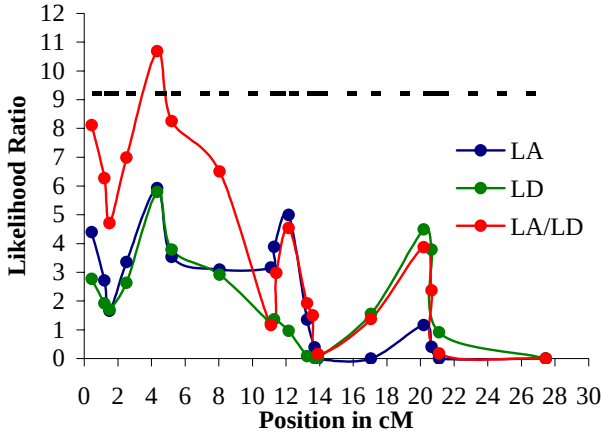

Figure 2. Restricted likelihood profiles of linkage equilibrium (blue), linkage disequilibrium (green) and combined linkage equilibrium/disequilibrium analysis (red). The dashed line shows a chromosome-wide significance threshold of LOD = 2. Line markings denote putative QTL positions.

examination of this chromosomal area should be conducted for verification and to pinpoint candidate genes in this area.

The random polygenic variance was estimated at 0.1002 and random gametic variance was estimated at 0.0092 at the putative QTL position in marker interval 5. The QTL variance (calculated as twice the gametic variance) was 0.00184; the ratio of QTL to polygenic variance was estimated at 0.155, with a RLRT statistic of 10.61. These results indicate that the QTL found in this study is responsible for 15.5% of the genetic variance in the trait SCS. Rest variance was estimated at 0.0972.

A second goal of the study was to identify haplotypes associated with increases or decreases in mastitis resistance in the population.

Each unique haplotype effect was estimated in our study; the paternal and maternal effects of a particular animal can be summed to obtain the total estimated effect for the animals' genotype. Figure 3(a) displays the distribution of genotype (QTL) effects at marker interval 5 as the sum of both haplotypes of an individual divided by the corresponding standard error of the sum. Values ranged from -1.77 to 1.19 (average = -0.11, standard deviation = 0.51). Animals with negative values (left side of graph) are likely to be QTL homozygous with a positive effect on SCS, whereby animals with positive values show an increase in SCS (right side of graph) and can be considered likely homozygous with an unfavourable effect. The animals from the extreme left and right of the graph (with the lowest and highest genotype effects) could be chosen for comparative sequencing analyses. Similarly, the absolute difference between estimated haplotype effects were analysed in order to determine likely QTL heterozygotes (on the far right of Figure 3(b)).

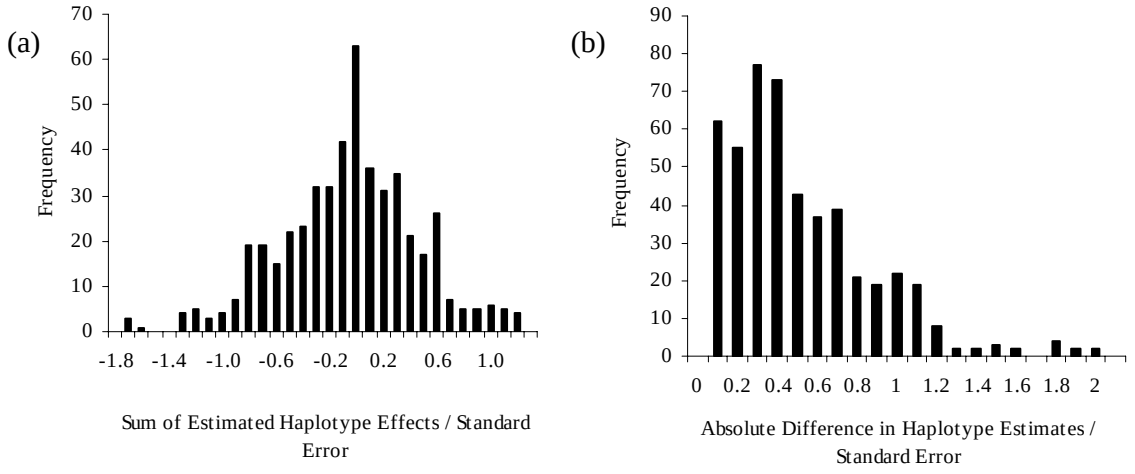

Figure 3. Frequency distribution of the sums (a) and differences (b) of estimated genotype effects at the fifth marker interval (4.34 cM) divided by the standard error.

CONCLUSIONS

A combined LD/LE analysis was used to fine map a QTL affecting SCS in the German Holstein population. This QTL is responsible for 15.5% of the genetic variation in the trait SCS. Furthermore, linkage disequilibrium was found across all 6 families studied, providing evidence that the chromosomal area on BTA27 under investigation may be a prime candidate for use in a MAS breeding program. Finally, specific haplotypes associated with both increases and decreases in SCS could be identified. These results provide a very promising step towards incorporating udder health traits such as SCS into MAS breeding programs.

ACKNOWLEDGEMENTS

The authors would like to thank the Federation of German Cattle Breeders (Arbeitsgemeinschaft Deutscher Rinderzüchter, ADR) for their supply of data. Financial support from the FUGATO.M.A.S.-Net project is gratefully acknowledged. The EAAP is also appreciatively acknowledged for scholarship funds.

REFERENCES

BAES C., REINSCH N.:
Computing the condensed conditional gametic QTL relationship matrix and its inverse. Arch. Tierz. **50** (2007) 3:294-308

GILMOUR A., GOGEL B., CULLIS M., THOMPSON R.:
ASReml User Guide Release 2.0. (2006) VSN International Ltd., Hemel Hempstead, HP1 1ES, UK

KHATKAR M., THOMSON P., TAMMEN I., RAADSMA H.:
Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genet. Sel. Evol. **36** (2004) 163-190

LIU Z., REINHARDT F., BÜNGER A., REENTS R.:
Derivation and Calculation of Approximate Reliabilities and Daughter Yield Deviations of a Random Regression Test-Day Model for Genetic Evaluation of Dairy Cattle. (2004) J. Dairy Sci. 87:1896-1907.

MEUWISSEN T., GODDARD M.:
Prediction of identity by descent probabilities from marker haplotypes. Genet. Sel. Evol. **33** (2001) 605-634

REINSCH, N.:
A multiple-species, multiple-project database for genotypes at codominant loci. J. Anim. Breed. Genet. **116** (1999) 425-435

TUCHSCHERER A., MAYER M., REINSCH N.:
Identification of gametes and treatment of linear dependencies in the gametic QTL-relationship matrix and its inverse. Genet. Sel. Evol. **36** (2004) 621-642