A two step approach to detect interaction between QTL and a known majorgen in dairy cattle

D. Hinrichs[*], J. Bennewitz[†], *J. Tetens*[*] , N. Reinsch[‡] and G. Thaller[*]

Introduction

In theory genetic variation could be divided in different components due to additive, dominance, and interaction effects of genes. Hill et al. (2008) postulated that most of the genetic variance is additive, which is a consequence of a U-shaped distribution of gene frequencies with most near 0 or 1. On the other hand, Carlborg and Haley (2004) pointed to the importance of epistatic effects in QTL mapping. Their reasoning is mainly based on experimental crosses, where QTL gene frequencies are more or less intermediate in the final cross. For example, Carlborg et al. (2004) reported that the amount of variance which could be explained by epistatis ranged between 19% and 31% in a Jungle fowl x White Leghorn F_2-intercross. In outbred populations only very limited is known about mapped QTL showing epistatic effects. Main reason for this is the limited power to detect epistasis in classical QTL-experiment designs, e.g. half-sib designs. If however, a gene would be known or at least a marker being in strong linkage disequilibrium with the gene, power to detect epistatic effects of further QTL with this gene could be increased.

The aim of this study was to search for epistatic effects between QTL for protein percentage and fat percentage and the known *DGAT1* gene in the German Holstein dairy cattle population using a two step approach.

Material and methods

Animals.
The pedigrees utilized were part of the granddaughter design of a QTL mapping project in Germany, which was described in detail by Thomsen et al. (2000). It included 16 German Holstein families with a total of 872 progeny tested bulls. The family size varied between 19 and 127 with an average of 54 sons.

Genotypes and Phenotypes
The families were genotyped for approximately 250 different markers, which were mainly microsatellite markers. The average number of marker loci per chromosom was 8.8 and ranged from 2 to 15. A more detailed description of the marker and the genetic map is given by Thomsen et al. (2000). *DGAT1* (Winter et al. 2002, Grisart et al 2002) was also

[*] Institute of Animal Breeding and Husbandry, Christian-Albrechts-University, Hermann-Rodewald-Straße 6, 24118 Kiel, Germany
[†]Institute of Animal Husbandry and Breeding, University Hohenheim, D-70599 Stuttgart, Germany
[‡]Leibniz Institute for Farm Animal Biology Unit Genetics and Biometry, Wilhelm Stahl Allee 2, D-18196 Dummerstorf, Germany

genotyped, the genotypes were coded as g = Alanin/Alanin, Alanin/Lysin, and Lysin/Lysin, respectively (g = A/A, A/L, and L/L). The effects of this gene was shown to be highly significant in the Germans Holsteins, with Lysin being the allele increasing milk protein content and milk fat content (Thaller et al. 2003, Bennewitz et al. 2004). The estimated breeding values were taken from the routine national sire evaluation in November 2009. The mean breeding value for protein percentage was -0.02 and the estimates ranged from -0.43 to 0.30. The estimated breeding values for fat percentage varied between -0.77 and 0.78 and the mean breeding value was 0.02.

Statistical analyses

The statistical analysis of this study was done in two steps. The first step was a genomescan to detect QTL using a multiple regression. For each cM on the chromosome following model was applied:

$$y_{ij} = g_{si} + b_1 \cdot x_{ij} + b_{ik} \cdot tp_{ijk} + e_{ijk}, \quad [1]$$

where y_{ij} is the phenotype of *j*th sire (being a son of grandsire i), gs_i is the fixed effect of the *i*th grandsire, x_{ij} is the number of lysine alleles (0, 1, or 2) carried by the sire ij, b_1 is the corresponding regression coefficient, tp_{ijk} is the QTL transition probability of sire ij at the *k*th chromosomal position, b_{ik} is the regression coefficient for the *i*th grandsire at the *k*th chromosomal location, and e_{ijk} is the random residual. The null hypothesis tested with this model is that no QTL segregated on a particular chromosome. The alternative hypothesis is that one QTL segregated on the chromosome. To account for multiple testing, chromsomewise test statistic critical values were calculated using the permutation test (Churchill and Doerge, 1994), performing 10,000 permutations. The calculations were done with the software BIGMAP and ADRQLT (Reinsch, 1999).

In the second step the following model was applied at those chromosomal positions were a significant QTL was mapped using the model shown above. The model was

$$y_{ij} = g_{si} + b_1 \cdot x_{ij} + b_{igk} \cdot tp_{ijk} + e_{ijk}, \quad [2]$$

where the regression on tp was performed within grandsire i and *DGAT1* genotype (g) of the sires. Hence, up to three QTL regression coefficients were estimated for each grandsire family (instead of only one in model [1]). The null hypothesis was that within a grandsire family every estimated regression coefficient were the same, the corresponding alternative hypothesis was that at least two regression coefficient differ from each other in at least one familiy. The alternative hypotheis was accepted if the comparisonwise error probability was below 0.05. No correction for multiple testing was necessary because the model was only applied to significant QTL positions.

The rejection of the null hypothesis can be interpreted as an epistatic effect between the QTL at this position and *DGAT1* for the following reason. Among daughters of a *DGAT1* heterozygous (homozygous) sire all three (only two) *DGAT1* genotypes can be found. The gene frequencies between daughter groups of sires with different *DGAT1* genotypes differ. This difference is one quarter between the daughter groups of a *DGAT1* homozygous and a *DGAT1* heterozygous sire and one half between those of two alternative *DGAT1*

homozygous sires. Hence, any interaction effects between *DGAT1* and a QTL is due to the difference in *DGAT1* gene frequencies of these daughter groups. EBVs were used as phenotypes of the sires and these EBV are determined mainly by the daughter performance. It is assumed that these EBV contain a part of the interaction effects, which are most likely additive by additive interaction effects (Falconer and Makay 1996).

Results and discussion

Table 1 shows the significant QTL for protein percentage (results from model (1)) and the test for interaction effects with *DGAT1* (results from model (2)). Five chromsomewise significant QTL were found, four of them showed significant epistatic effects with *DGAT1*.

Table 1: Test statistic and error probabilities for mapped QTL (results from model one) and QTL by *DGAT1* epistatic effects (results from model two) for protein percentage.

BTA	Position	F	$p_{chromosomewise}$	$F_{interaction}$	$p_{interaction}$
14	54	2.98	0.0008	1.39	0.0980
05	113	2.24	0.0308	1.60	0.0322
17	76	2.22	0.0324	1.11	0.3247
06	53	2.14	0.0416	0.86	0.6603
15	70	2.09	0.0424	1.21	0.2175

Table 2 gives a more detailed description of the interactions, i.e. which pair of regression coefficient differed within grandsire family. The significance was due to the difference between the regression coefficients of the alternative homozygous *DGAT1* genotypes.

Table 2: Significance test for different QTL regression coefficients among *DGAT1* genotypes, results from model two.

BTA	Pos.	$F_{AA\ vs.\ AL}$	$P_{AA\ vs.\ AL}$	$F_{AA\ vs.\ LL}$	$P_{AA\ vs.\ LL}$	$F_{LL\ vs.\ AL}$	$P_{LL\ vs.\ AL}$
14	54	1.31	0.1855	2.21	0.0199	0.93	0.5005
05	113	1.05	0.4025	2.97	0.0017	1.32	0.2202

The results for fat percentage can be found in Table 3 and 4. Five significant QTL could be found and three of them interacting with *DGAT1*. The significance of the interaction between the QTL and *DGAT1* was due to the heterozygote sires and the homozygote LL sires (see Table 4).

Table 3: Test statistic and error probabilities for mapped QTL (results from model one) and QTL by *DGAT1* epistatic effects (results from model two) for fat percentage

BTA	Pos.	F	P chromosomewise	F interaction	P interaction
14	18	3.46	< 0.0001	1.44	0.0765
05	112	2.66	0.0064	1.58	0.0355
29	56	2.44	0.0072	1.19	0.2395
19	76	2.45	0.0160	1.39	0.0974
15	80	2.30	0.0340	1.27	0.1715

Table 4: Significance test for different QTL regression coefficients among *DGAT1* genotypes, results from model two.

BTA	Pos.	$F_{AA\ vs.\ AL}$	$P_{AA\ vs.\ AL}$	$F_{AA\ vs.\ LL}$	$P_{AA\ vs.\ LL}$	$F_{LL\ vs.\ AL}$	$P_{LL\ vs.\ AL}$
14	18	1.07	0.3812	0.78	0.6317	2.42	0.0103
05	112	1.12	0.3309	2.44	0.0097	1.29	0.2366

Conclusion

Two of five significant QTL for protein percentage showed interaction with *DGAT1*. For fat percentage five significant QTL were found and two of them interacting with *DGAT1*. These results showed that (i) it is possible to detect epistatic effects using the two step approach and (ii) epistatsis is common for the majorgene *DGAT1* and significant QTL for these two traits. Further investigations are needed in order to fully understand these interaction effects.

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