Quantitative Trait Locus-by-Environment Interaction for Milk Yield Traits on *Bos taurus* Autosome 6

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ABSTRACT

Genotype-by-environment interactions for production traits in dairy cattle have often been observed, while QTL analyses have focused on detecting genes with general effects on production traits. In this study, a QTL search for genes with environmental interaction for the traits milk yield, protein yield, and fat yield were performed on *Bos taurus* autosome 6 (BTA6), also including information about the previously investigated candidate genes ABCG2 and OPN. The animals in the study were Norwegian Red. Eighteen grandsires and 716 sires were genotyped for 362 markers on BTA6. Every marker bracket was regarded as a putative QTL position. The effects of the candidate genes and the putative QTL were modeled as a regression on an environmental parameter (herd year), which is based on the predicted herd-year effect for the trait. Two QTL were found to have environmentally dependent effects on milk yield. These QTL were located 3.6 cM upstream and 9.1 cM downstream from ABCG2. No environmentally dependent QTL was found to significantly affect protein or fat yield.

SEVERAL studies have reported genotype-by-environment interactions for milk production traits in dairy cattle, where the environment is measured as the average herd production level (CALUS *et al.* 2002; KOLMODIN *et al.* 2002). This implies that some genes have different effects according to the environment. These environmentally dependent gene effects can be of physiologic interest and the detection of such genes might have practical consequences for the breeding program. Environmentally dependent gene effects can be detected by including an interaction term between a putative QTL and the environment in the QTL search. In this article a method of QTL mapping is used that allows the QTL effect to change gradually over a continuous environmental scale.

In cases where the environment is easily categorized into distinguishable classes, a multiple-trait approach may be used to include QTL-by-environmental interaction in the model. Alternatively, where the environment is a continuous variable, random regression models have been used to describe genotype-by-environment interactions (DE JONG 1995) and later extended to include QTL-by-time interactions for longitudinal data (LUND *et al.* 2002) and QTL-by-environment interac-

tions (LILLEHAMMER *et al.* 2007a). Similar methods have also been shown to be effective for QTL detection in plants (KOROL *et al.* 1998; BOER *et al.* 2007). Random regression models measure the QTL effect as a function of a continuous environmental variable and are therefore well suited for analyses where the environment is measured on a continuous scale (KOLMODIN *et al.* 2002). In the case of QTL-by-environment interaction, using a random regression model might increase the power of QTL detection, compared to a model that ignores the QTL-by-environmental interaction, especially if the QTL has only a small average effect but large effects in some environments (LILLEHAMMER *et al.* 2007a).

The detection of environmentally dependent genes may increase the understanding of the biology behind genotype-by-environment interaction. These genes also represent an opportunity to change environmental sensitivity through selection. If genes with environmentally dependent effects are implemented in selection, without taking the environment into account, the selection response might be poor or negative in certain environments. A challenge is how to define the environment. Production level has been a preferred environmental variable because it has the ability to capture a complex environment into a single variable (CALUS *et al.* 2002; KOLMODIN *et al.* 2002). In this study, production level was defined by the predicted herd-year effect on the trait.

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Bos taurus autosome 6 (BTA6) has been found to harbor one or more QTL affecting production traits in several dairy cattle populations (*e.g.*, RON *et al.* 2001; FREYER *et al.* 2002; OLSEN *et al.* 2002). Other studies have reported possible candidate genes for these QTL. Both SCHNABEL *et al.* (2005) and LEONARD *et al.* (2005) pointed out OPN as a strong candidate. SCHNABEL *et al.* (2005) identified a deletion upstream of the OPN promoter (OPN_3907), in a region known to harbor tissue-specific osteopontin regulatory elements, which was in complete concordance with the segregation status of bulls segregating for the QTL. Several SNPs have been identified in the ABCG2 gene, of which the most focused is an A to C substitution in exon 14, causing a change of the amino acid from tyrosine to serine (COHEN-ZINDER *et al.* 2005; OLSEN *et al.* 2005). This SNP was first reported, and called ABCG2_49, by OLSEN *et al.* (2005). COHEN-ZINDER *et al.* (2005) found that the mutation affected milk yield and protein and fat percentages and further suggested that more genes affecting milk yield were segregating on BTA6.

LILLEHAMMER *et al.* (2007b) performed a genome scan over all *B. taurus* autosomes except BTA6 for environmentally dependent QTL. The aim of this study is to search for possible gene-by-environment interactions for milk yield traits on BTA6. BTA6 is addressed separately for two reasons. This autosome, as mentioned earlier, has been shown to contain several QTL for milk production traits, and second a dense marker map was available for this autosome. The detected QTL and the effects of ABCG2_49 or OPN_3907 were characterized as environmentally dependent or environmentally resistant with special attention to the environmentally dependent QTL effects and possible applications of such QTL.

MATERIALS AND METHODS

All the animals in the study belonged to the Norwegian Red cattle breed. No genotypes from cows were available. Eighteen grandsires and 716 sires were genotyped for 362 markers on BTA6. The markers were 17 microsatellites described by OLSEN *et al.* (2005) and a subset of 345 SNPs taken from an earlier version of the dense SNP map for BTA6 described by NILSEN *et al.* (2008). Marker order and map distances were estimated using the CRI-MAP program 2.4 (GREEN *et al.* 1990) with map distances based on Haldane's mapping function.

Phenotypes from 2,295,982 cows consisted of milk yield (10³ kg), protein yield (kilograms) and fat yield (kilograms), precorrected for heterogeneous variance due to parity and age within parity. Corrected yields (cy) were analyzed with the official repeatability animal model, including the fixed effects of age within parity (age $\times$ par), month of calving within parity (cm $\times$ par), days open within parity (do $\times$ par), and year of calving (year), and for the random effects of herd year (hy), animal (genetic effect), and permanent environment of the cow (pe). hy is an environmental factor that describes in which herd and in what year the lactation started, assuming that herds and years provide different environments. Yields from three lactations were included in the repeatability model

analysis. The precorrections and the repeatability model analyses were the same as in the official Norwegian breeding value estimation. Details can be found at http://www.interbull.slu.se/national_ges_info2/framesida-ges.htm under "production" and "Norway."

Records for the subsequent QTL mapping analyses were obtained from the repeatability model by subtracting fixed and random effects from the yield,

$$Y = cy - \text{age} \times \text{par} - \text{cm} \times \text{par} - \text{do} \times \text{par} - \text{year} - \text{hy} - \text{pe}, \quad (1)$$

where Y is the record used in the QTL mapping analyses.

The prediction of the random herd-year solution (hy) was used as the variable describing the production level of the herd in the subsequent QTL mapping analyses. This is a continuous variable that accounts for all factors that affect the production (CALUS *et al.* 2002). It allows for the herds to be put on a scale from "low-yielding environment" to "high-yielding environment." Only first lactation records from daughters of genotyped bulls (597,613 cows) were included in the subsequent QTL mapping analyses. Including more lactations in the repeatability model gives better predictions of herd-year effects and decreases the correlation between the herd-year effects and the corrected yields (KOLMODIN *et al.* 2002).

Linkage phases of grandsires and sons were estimated on the basis of marker information. The midpoint of each marker bracket was regarded as a putative position for a QTL, and the identical-by-descent (IBD) probabilities of pairs of haplotypes were calculated from marker and pedigree information by combined linkage disequilibrium and linkage analysis, using all 362 markers (MEUWISSEN *et al.* 2002). The haplotypes were correlated. The correlation between two different haplotypes refers to their IBD probability. Markers with an estimated distance of 0.0 cM were given a distance of 0.001 cM for computational reasons. Effective population size was assumed to be 250, and number of generations since mutation was assumed to be 100 (MEUWISSEN *et al.* 2002).

The performance of each daughter was modeled by a fixed regression on the random herd-year solution (predicted from the repeatability model), the random sire effect, and the interaction (random regression) between the sire effect and the herd-year solution (model 0). The regression on herd year was included even though it was already corrected for in a previous step to make sure that a possible remaining herd-year effect due to differences in data materials and models was not included in the interaction term:

$$Y_{ij} = \mu + bhy_{ij} + \alpha_i + \beta_i hy_{ij} + e_{ij}$$

(model 0), where Y_{ij} is precorrected first lactation milk yield, protein yield, or fat yield for daughter j of sire i (from Equation 1), μ is the overall mean, b is the fixed regression coefficient on hy_{ij} , and hy_{ij} is the herd-year solution of daughter j of sire i , obtained from the repeatability model. α_i is the random effect of sire i in the average environment, where $\text{Var}(\alpha) = A\sigma_\alpha^2$. A is the relationship matrix among sires, and σ_α^2 is the sire variance in the average environment. β_i is the random effect of sire i on the environmental sensitivity, *i.e.*, on the slope. $\text{Var}(\beta) = A\sigma_\beta^2$, where σ_β^2 is the sire slope variance. The covariance between intercept and slope, $\text{cov}(\alpha, \beta)$, was modeled as $A\sigma_{\alpha\beta}$, where $\sigma_{\alpha\beta}$ is the covariance between intercept and slope of the sire effects. e_{ij} is the random residual term of daughter j of sire i . All statistical analyses were performed by ASREML (GILMOUR *et al.* 2002).

Extending model 0 with single-gene effects made all other models. Table 1 describes the extensions. All QTL effects were assumed random with a variance in intercept equal to $G\sigma_{qi}^2$, where G is the IBD matrix of the haplotypes in the putative

TABLE 1
The extensions made to model 0 to build the other models

Model	ABCG2 ^a fixed		OPN ^b fixed		QTL ^c random		QTL2 ^d random	
	Int ^e	Slope ^f	Int ^e	Slope ^f	Int ^e	Slope ^f	Int ^e	Slope ^f
1a	x ^g	x ^g	x ^g	x ^g				
1b	x	x	x	x				
2a					x			
2b					x	x		
3a	x	x			x			
3b	x	x			x	x		
4a	x	x			x	x	x	
4b	x	x			x	x	x	x

^aThe fixed effect of the ABCG2_49 alleles.

^bThe fixed effect of the OPN_3907 alleles.

^cRandom effect of a putative QTL.

^dRandom effect of a second putative QTL.

^eThe effect of the gene in the average environment. Int, interaction.

^fThe interaction term between the gene and the herd-year solution, *i.e.*, the effect of the gene on the environmental sensitivity.

^gThe model was run twice, once with ABCG2 as the only single gene and once with OPN as the only single gene.

QTL position and σ_{qi}^2 is the variance in intercept due to this QTL. The slope variance due to the QTL was assumed to be $G\sigma_{qs}^2$, where σ_{qs}^2 is the QTL variance for slope, while the covariance between intercept and slope of the QTL effects was modeled as $G\sigma_{qi,qs}$, where $\sigma_{qi,qs}$ is the covariance between intercept and slope due to the QTL.

To check whether the mutations ABCG2_49 and/or OPN_3907 had an effect on milk yield, these markers were included as fixed effects in model 1, one at a time in model 1a, and both together in model 1b as two different fixed effects (Table 1).

To search for possible QTL, the daughter records were further analyzed using models 2a and 2b (Table 1), which are model 0 extended with a general QTL effect (model 2a) and a QTL effect on both intercept and slope as a random regression (model 2b). At the midpoint of each marker bracket, a likelihood-ratio test (LRT) of model 2b with model 0 as the reduced model was performed to test whether a significant QTL was segregating at that position. When model 2b gave a significant QTL ($P < 0.0001$), another LRT was performed where model 2b was tested against model 2a. The latter tests whether the interaction term is significant, given that there is a QTL in that position, *i.e.*, whether the detected QTL is environmentally dependent. The QTL was regarded as environmentally dependent if $P < 0.005$. The threshold was somewhat relaxed in this case, since only the significant positions were tested, so the number of tests was reduced.

For each LRT, the threshold value was obtained from the chi-square table. The degrees of freedom were assumed to be the numbers of extra parameters in the full model compared to the reduced model. For every new QTL fitted, three extra parameters were added in the model (intercept variance, slope variance, and covariance between intercept and slope). For every interaction test, there were two extra parameters in the full model, compared to the reduced model, the slope variance and the covariance between intercept and slope.

After concluding that ABCG2_49 had higher evidence of affecting milk yield than OPN_3907, the suggested environmentally dependent QTL were fitted one at a time, together with the fixed effect of ABCG2_49, to test whether they remained significant (models 3a and 3b). Model 3b was tested against the model including ABCG2_49 but no other single-

gene effects (model 1a) and against model 3a to check the significance of the interaction term.

Since the results indicated several environmentally dependent QTL, the most probable QTL were fitted pairwise in model 4b to avoid two positions linked to the same gene to be reported as two different QTL. In model 4b both positions include both a general effect of the QTL and a random regression of QTL effect on herd-year solution (Table 1). Model 4b was tested against model 3b for each of the two tested positions to test for significance of the second QTL, given ABCG2_49 and the first QTL. For the two most probable QTL, a LRT of model 4b with model 4a as the reduced model was used to test whether the second QTL was environmentally dependent. Only the QTL that obtained significance on a stringent significance level (<0.0001) with model 4b, compared to 3b, was reported as significant.

Model 0 gives estimates of the sire variance (genetic) split into variance in the average environment (intercept) and variance in environmental sensitivity (slope). On the basis of the results from model 4b, the contribution from each gene to the genetic variance was estimated. For ABCG2, the variance was estimated from the parameter estimates, while for the two QTL, the variance estimated in ASREML was doubled because each individual carries two QTL alleles. Since all single-gene effects and the total genetic effect were estimated on a sire level, the variances were comparable. The sire variance obtained from model 0 was used as an estimate for the total genetic variance. The variances of the single genes were presented as percentages of the total genetic variance for intercept and slope, respectively.

RESULTS

The analyses of the full data set with models 2a and 2b for all positions and traits gave several peaks for all three traits (Figures 1–3). The positions of some of the markers and other known genes are indicated as dots in Figures 1–3. A major peak at ~60 cM and a following smaller peak around the casein gene position were

FIGURE 1.—Likelihood-ratio curves of milk yield with and without interaction with herd-year–milk-yield solution. In addition to a peak at ~60 cM, which does not show interaction, six significant peaks ($P < 0.0001$) with significant interactions ($P < 0.005$) were found. Those are indicated with circles. Some common genes and markers are indicated as dots on the x -axis.

present for all traits, although not significant for fat yield. In these positions there was hardly any difference in LRT value between model 2a and model 2b. One or two environmentally resistant QTL seemingly exist in this area for protein yield and milk yield. Milk yield was the only trait to obtain significant results with a $P < 0.0001$ for the presence of a QTL and a $P < 0.005$ for the interaction term between QTL and environment of the detected QTL. For milk yield, six environmentally dependent QTL were found to be significant on this level (Figure 1). Their exact positions are given in Table 2. This gave six suggested QTL, all affecting milk yield.

When fitting either ABCG2_49 or OPN_3907 in the model (model 1a), significant F -values were obtained

for both intercept and slope (Table 3). Including both OPN_3907 and ABCG2_49 in the model showed that both the candidate genes get low F -values when the other gene is fitted in the model (Table 3). Fitting one of the candidate genes as fixed effects seems to be able to pick up the effect explained by both. ABCG2_49 was chosen as a cofactor in the remaining analysis, since it had the highest initial F -value (Table 3). The results indicate that ABCG2_49 has almost no effect in an extreme low-yield environment and that the gene effect increases when the environment improves, *i.e.*, with increasing production level.

Including ABCG2_49 in the model (models 3a and 3b) resulted in decreased LRT statistics for all the

FIGURE 2.—Likelihood-ratio curves for protein yield with and without interaction with herd-year–protein yield. Three peaks were found close to the casein complex, but none of the peaks showed significant interaction. Some common genes and markers are indicated as dots on the x -axis.

FIGURE 3.—Likelihood-ratio curves for fat yield, with and without interaction. Some common genes and markers are indicated as dots on the x -axis. No significant QTL for fat yield with interaction with herd-year–fat-yield solution was found.

suggested QTL (Table 2). However, it did not remove the evidence of another QTL completely, indicating that ABCG2₄₉ is not the cause of all the QTL variation on BTA6. The three QTL that got the highest LRT values when model 3b was tested against 1a, hereby referred to as confirmed QTL, were fitted pairwise in model 4b; the QTL in position 41.8 cM remained significant when fitted together with one of the other two confirmed QTL (Table 4). The other two QTL (in positions 21.8 and 29.2 cM) were not significant when fitted in the same model (Table 4). Fitting the two most probable positions (29.2 and 41.8 cM) showed that they both seem to be environmentally dependent, since the interaction term between each QTL and the environment remained significant also in the two-locus model (Table 4).

Figure 4 shows the predicted solutions of the grand-sire's haplotypes for the two QTL in positions 29.2 and 41.8 cM. The QTL effect is shown as a function of the environmental descriptor hy . Figure 4 shows how the

haplotypes differ both in their average effect on milk yield and in environmental sensitivity for milk yield.

The results from model 4b imply that ABCG2₄₉ contributes 1.89% of the total genetic variance for intercept, which was estimated to be 0.2614, and 2.16% of the total genetic variance for slope, which was estimated to be 0.01197. The QTL in position 29.2 cM contributes 4.77% of the total genetic variance for intercept and 34.9% of the total genetic variance for slope. The QTL in position 41.8 cM contributes 7.82% of the genetic intercept variance and 11.2% of the genetic slope variance.

DISCUSSION

All the six suggested environmentally dependent milk yield QTL were found within a region of ~ 35 cM of BTA6 (Figure 1). Several studies have reported QTL for milk yield within this region. FREYER *et al.* (2002) reported a QTL close to BM1329. On our marker

TABLE 2
LRT statistics of the different likelihood-ratio tests of the full data set

Position (cM)	LRT _(2b vs. 0)	LRT _(2b vs. 2a)	LRT _{(3b vs. 1a)^a}	LRT _(3b vs. 3a)
21.8	23.94***	23.60***	14.08*	14.08**
29.2	35.38***	24.14***	21.66***	17.46**
34.3	26.12***	13.02*	8.12	1.92
40.2	22.02***	13.78*	6.16	1.98
41.8	28.4***	15.54**	19.32**	8.66
54.5	22.28***	18.54***	2.48	0.22

The given position is the distance from the first marker on the map (ANXA5_1518). *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.005$.

^aModel 3b tested against model 1a, including the fixed effect of ABCG2₄₉.

TABLE 3
F-values of the candidate genes

Intercept	Slope
When fitting ABCG2_49 only with model 1a	
$F = 7.88$	$F = 8.26$
When fitting OPN_3907 only with model 1a	
$F = 5.50$	$F = 5.44$
OPN intercept	OPN slope
Fitting ABCG2_49 and OPN_3907 together in model 1b	
$F = 1.60$	$F = 1.28$
ABCG2 intercept	ABCG2 slope
Fitting ABCG2_49 and OPN_3907 together in model 1b	
$F = 4.10$	$F = 3.83$

F-values from fitting ABCG2_49 and/or OPN_3907 as fixed effects in the model are shown.

map, BM1329 is only 0.7 cM away from the suggested QTL in position 21.8 cM (Figure 1). RON *et al.* (2001) reported a QTL with BMS2508 as the closest marker, and that is in the same area where the QTL in position 29.2 cM of this study was found (Figure 1). KUHN *et al.* (1999) reported a QTL close to FBN13, which is close to the QTL in position 41.8 cM in this study (Figure 1). Several studies have reported QTL close to BM143 (*e.g.*, VELMALA *et al.* 1999; OLSEN *et al.* 2002), and ABCG2 (COHEN-ZINDER *et al.* 2005) and OPN (SCHNABEL *et al.* 2005) have been suggested as possible explanations for this QTL. The results of this study indicate that some of the previously reported QTL might have environmentally dependent effects.

The distances between these findings are quite small (the distance between BM1329 and FBN13 on our map is 22.2 cM), and confidence intervals for reported QTL tend to be large, although smaller when linkage disequilibrium information is included. ABCG2 is located between BM1329 and FBN13 and could be the cause of all the reported QTL in this area. However, the

TABLE 4
LRT statistics from testing model 4b (containing two random QTL, both environmentally dependent) against model 3b (containing only one random environmentally dependent QTL)

Position (cM)	Position of the first QTL fitted (cM)		
	21.8	29.2	41.8
21.8		3.10	27.30
29.2	10.68		35.82 (18.02) ^a
41.8	32.54	33.48 (10.64) ^a	

^aLRT statistic from testing model 4b against model 4a, to check the second QTL for environmental dependency after a first environmentally dependent QTL is fitted.

FIGURE 4.—The predicted solutions of the haplotypes of the two detected environmentally dependent QTL. Only haplotypes represented in the grandsires are presented. Each haplotype solution is presented as a linear function of the environmental descriptor herd year, to show how the haplotypes differ both in their general effect on milk yield and in their environmental sensitivity.

results of this study indicate that multiple QTL exist in this area. ABCG2 or OPN seems to affect milk production, but including ABCG2 as a cofactor does not eliminate the evidence of all the other QTL. Meta-analysis of the results from several studies has concluded that BTA6 harbors more than one milk-yield QTL (KHATKAR *et al.* 2004). The meta-analysis concluded that one significant QTL was found close to BM143 and another one was found close to the casein position. FREYER *et al.* (2003) also found two different QTL on BTA6 affecting milk yield, one on each side of BM143. The positions of these two QTL are very close to the QTL in positions 29.2 and 41.8 cM in this study; however, one of them might be ABCG2 or OPN. Among these previously reported QTL, it seems as if QTL caused by or located close to ABCG2 have environmentally dependent effects, while QTL close to the casein position have environmentally resistant effects on milk yield.

While environmentally resistant QTL have a consistent effect, environmentally dependent QTL have an effect that changes over the environmental scale. Significant QTL were found for both milk yield and protein yield. The protein-yield QTL were both environment resistant, while several of the milk-yield QTL were dependent on the environment. Environmentally resistant QTL are expected to be easier to detect and to give more robust estimates of QTL effects among studies. They might also be better suited for

marker-assisted selection, since the selection response can be predicted without extensive knowledge about the future production environment. The environmentally dependent QTL might be of great importance in some environments, although less important in other environments. In addition to affecting the average performance of the animals it affects how the animals react to changes in environmental conditions. This reaction, known as environmental sensitivity or reaction norm, might be an important trait for selection, and the environmentally dependent QTL facilitate selection for environmental sensitivity. The large proportion of the genetic variance in environmental sensitivity explained by the detected QTL implies that such selection could be done quite efficiently. However, the reported QTL variances are likely to be overestimated (BEAVIS 1994).

A stringent significance level was chosen to report QTL with strong evidence of existence only. Even though six positions were significant when fitted alone (with model 2), further analyses were required to distinguish between QTL positions and positions in linkage disequilibrium with a QTL.

Since ABCG2 and OPN both were suggested as candidate genes to affect milk production, some of the significant positions could be significant because of linkage disequilibrium with ABCG2 or OPN. In that case, including ABCG2 or OPN in the model should remove the effect of other QTL. However, including a fixed marker in the model may remove evidence for several QTL, both true and false, since the marker is in linkage disequilibrium with the other positions on the same chromosome.

Including ABCG2 decreased the LRT values for all suggested QTL, but three QTL got high LRT values after ABCG2 was included, although not necessarily significant on the same stringent significance level (Table 2). After fitting them pairwise into model 4, it was shown that the two QTL in positions 21.8 and 29.2 cM removed the evidence of each other (Table 4). These two are located close to each other, and they are probably in linkage disequilibrium with the same gene. The QTL in position 41.8 cM was not eliminated by any of the other two and did not eliminate evidence for any of the other two QTL. The results indicate that two environmentally dependent genes are segregating on BTA6, in addition to ABCG2 or OPN. These two genes are located on each side of ABCG2, only 3.6 and 9.1 cM, respectively, away from ABCG2, assuming that the QTL upstream from ABCG2 is located in position 29.2 cM, which had higher LRT values in all tests than position 21.8 cM.

The environmental interaction of ABCG2_49 shows higher variance due to the QTL when environment is improving. This was also the case of the QTL in position 29.2 cM (Figure 4). This is consistent with genotype-by-environment interaction studies that find that the genetic variance increases when the environment is improved (KOLMODIN *et al.* 2002; FIKSE *et al.* 2003).

Since both ABCG2_49 and the detected QTL in positions 29.2 and 41.8 cM affect milk yield without affecting protein yield or fat yield, it seems that they affect the amount of water that is supplied to the milk. This is confirmed for ABCG2_49 by COHEN-ZINDER *et al.* (2005) by finding that the allele that increases milk yield decreases protein and fat percentages. Water can be added to the milk by increasing lactose synthesis, since lactose is the most important osmotic component in the milk. The interaction pattern of ABCG2 implies that the allele causing high milk volume has greater effect in an environment that supports high average yield than in an environment where average milk yield is low. A possible explanation for this is that an adequate nutrition level is required for the animal to be able to utilize its genetic potential for lactose synthesis and hence that genes that affect the milk volume tend to be environmentally dependent. The detected QTL close to the casein position affect both milk yield and protein yield, and these QTL seem to be environmentally resistant. However, as genotype-by-environment interactions have been found for protein production in other studies (CALUS *et al.* 2002; KOLMODIN *et al.* 2002), environmentally dependent genes for protein are likely to be found as well. They might be located on other chromosomes (LILLEHAMMER *et al.* 2007b).

Two QTL were found to have environmentally dependent effects on milk yield when the candidate gene ABCG2 was included as a cofactor in the analysis. The QTL were located 3.6 cM upstream and 9.1 cM downstream from the ABCG2 gene and 29.2 and 41.8 cM from the first marker. When fitted in a model together with ABCG2_49 and the other QTL, both the QTL were significant on a 0.0001 nominal significance level, and both the QTL showed significant interaction with the environment ($P < 0.005$). These environmentally dependent genes will show inconsistent results when analyzed under different environmental conditions, and knowledge about the future environment is needed when deciding if the genes should be included in a selection scheme. Detected QTL close to the casein position, however, showed environmentally resistant effects for both milk yield and protein yield.

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