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A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayrshire cattle

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Summary

Cattle chromosome 6 was scanned with 11 markers, ten microsatellites and the casein haplotype, to identify quantitative trait loci (QTLs) affecting the following milk production traits: milk yield, fat percentage, fat yield, protein percentage and protein yield. Twelve Finnish Ayrshire half-sib families with a total of 480 sons were genotyped and used in a grand-daughter design. Interval mapping was performed with a multiple-marker regression approach with a one-QTL and a two-QTL model, and the significance threshold values were determined empirically using a permutation test. Across-family analysis with the one-QTL model revealed an effect on protein percentage ($P < 0{\cdot}05$) and on milk yield ($P < 0{\cdot}05$). The analysis with the two-QTL model identified significant effects ($P < 0{\cdot}05$) on protein percentage, milk yield, and fat yield. Comparing these two cases, the results suggest the existence of two QTLs on chromosome 6 with an effect on milk production traits. One of the QTLs was located around the casein genes. As the other QTL was similar in location and effect to a QTL found previously in Holstein–Friesians, an identity-by-descent approach could be applied to fine map this region.

Keywords: microsatellite, casein, interval mapping, regression, quantitative trait loci, cattle

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Introduction

Milk production traits in dairy cattle are quantitative in nature. The phenotypes observed are thus the combined results of the action of many genes or quantitative trait loci (QTLs) and environmental factors. Despite the complex nature of milk production traits, associations between them and individual genetic markers, casein genes on chromosome 6 in particular, have been widely studied (e.g. Ng-Kwai-Hang *et al*. 1990; Bovenhuis *et al*. 1992). Findings on the effects of casein genotypes on milk production traits vary, however, among different studies. Bovenhuis *et al*. (1992) postulated several reasons for the discrepancies: the studies were undertaken on different breeds; the statistical models and the alleles studied, especially for β-casein, differed; and the effects seen for variants at one casein locus could equally well have been due to a very closely linked gene(s), e.g. other casein loci. Application of the grand-daughter design of Weller *et al*. (1990) has recently gained currency in efforts to analyse associations between markers and milk production traits especially as the design enables a bull's breeding value to be assessed with much higher accuracy than in studies of individual cow records for a specific trait. Studies conducted using this approach have reported the significant effect of a casein haplotype within a grandsire family on milk and protein yield in Norwegian Red cattle (Lien *et al*. 1995) and on milk yield and fat percentage in Finnish Ayrshire (Velmala *et al*. 1995). Kühn *et al*. (1996) reported associations between milk production traits and different markers of chromosome 6 in three Holstein–Friesian families.

Due to advances in statistical methods and the collection of genetic markers on linkage maps it is now possible to scan the entire cattle genome for the existence of QTLs. In such a scan the use of multiple-marker instead of single-marker approaches has been shown to increase the power of the test and to give an estimate of the QTL location (Knott *et al*. 1996). Multiple-marker mapping of QTLs in half-sib populations can be performed using either maximum-likelihood (Georges *et al*. 1995) or least-squares approaches (Knott *et al*. 1996). Georges *et al*. (1995) found QTLs for milk production traits on chromosomes 1, 6, 9, 10 and 20 within American Holstein cattle. In an across-family study employing the least-squares approach, Spelman *et al*. (1996) detected a putative QTL on chromosome 6 affecting milk

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protein percentage in Dutch Holstein–Friesian cattle.

In the present study we searched for QTL on chromosome 6 associated with five milk production traits. Our aim was to locate more precisely the earlier association found with casein haplotypes (Velmala *et al.* 1995). In addition to casein haplotypes, the entire chromosome 6 was scanned with ten microsatellite markers. The single-marker analysis employed in our previous study was replaced by interval mapping using multiple-markers with the least-squares approach. Both one-QTL and two-QTL models were fitted. The analysis enabled us to identify putative QTLs affecting milk production traits in Finnish Ayrshire cattle.

Materials and methods

Family material

Twelve half-sib families containing a total of 480 artificial insemination (AI) bulls from Finnish Ayrshire dual-purpose cattle were used in the grand-daughter design of Weller *et al.* (1990). Seven of these families were same as in our previous study (Velmala *et al.* 1995), except the number of sons/family was higher in the present study. In addition five new families (35076, 36022, 36378, 36386, and 36455) were analysed. The oldest families, especially 33090 and 33787, had semen available only for selected sons and therefore contain a biased sample of sons. Quantitative trait scores for milk yield, fat percentage, fat yield, protein percentage and protein yield were obtained from the national cow evaluation of May 1996. The best linear unbiased prediction (BLUP) of a breeding value by animal model was used for each son. Although it would have been more correct to use the daughter yield deviations, we opted for the BLUP values since all the sons had well over 100 daughters (except for 11 sons that had 70–95 daughters). With such high numbers of daughters, the difference between daughter yield deviations and BLUP values becomes negligible for high heritability traits.

DNA analysis of markers

The semen samples for each son were provided by five Finnish AI societies. The DNA was extracted from semen samples as described in Zadworny & Kuhnlein (1990) and distributed into 96-well microtiter plates. Markers were chosen to span chromosome 6 according to maps of Barendse *et al.* (1994) and Bishop *et al.* (1994). Additional sources of microsatellite primer sequences were Jorgensen *et al.* (1995) and Kemp *et al.* (1995). The casein genes α_{S1}-, β -, and κ-casein, were considered jointly as one marker, with haplotypes being treated as alleles. A microsatellite within the κ-casein gene, the κ-casein *A*, *B*, and *E* alleles, the β-casein A^1 and A^2 alleles, and the α_{S1}-casein *B* and *C* alleles were genotyped as explained in Velmala *et al.* (1995; and references therein). All microsatellite primers were synthesised on an Applied Biosystems 392 DNA synthesizer (Foster City, CA). One primer for each locus was fluorescein-labelled during synthesis (FluorePrime, Pharmacia, Uppsala, Sweden). All PCR reactions for microsatellites (volume 25 μl) contained 200 μM each dNTP, DyNAZyme^{TM} buffer with 1·5 mM $MgCl_2$ (Finnzymes, Espoo, Finland), 1 U of DyNAZymeII DNA polymerase (Finnzymes), and 50 ng of DNA template. The primer concentrations varied from 0·2 μM to 1·0 μM and the primers for *ILSTS93* and *ILSTS97*, *RM28* and *BM1329*, and *ILSTS87* and *BM4528* were multiplexed in a PCR reaction. All DNA amplifications were performed in a PTC-100 Programmable Thermal Cycler (MJ Research Inc., MA) as follows: 4 min at 94 °C, 26 cycles (30 for *BP7*, *ILSTS93*, *ILSTS97* and *AFR227*) of 30 s at 94 °C, 30 s at 55 °C (58 °C for *BP7*, *RM28* and *BM1329*; 66°C for *AFR227*) and 30 s at 72 °C, and finally 6 min at 72 °C. The PCR products were separated on 6% denaturing PAGE gels (ReadyMix, Pharmacia) using the A.L.F.^{TM} DNA Sequencer (Pharmacia). For size determination an internal size standard (SIZER; Pharmacia) was included in each lane. The gels were analysed using the Fragment Manager V1·1 programme (Pharmacia). Potential genotyping errors were checked by reanalysing all genotypes leading to double and multiple cross-overs following construction of the genetic map for chromosome 6.

Statistical analysis

The map distances between the loci were estimated from our data using ANIMAP program as described in Georges *et al.* (1995), which converts the recombination fractions between two loci to map distances with the Haldane mapping function assuming no interference. Because no recombinants were detected between *ILSTS87* and *BP7*, we combined the information from these two loci resulting in 11, not 12, marker positions in the interval mapping analysis. In that analysis only the sons having both the phenotype and unambiguous genotype data were included. In two families only one son had inherited the

alternate paternal allele for one marker. These cases were omitted from the analysis (see Table 1), because the phenotypic mean of that marker class would have been based on only one individual. The information content along chromosome 6 measured by the variance of QTL conditional probability was calculated following Spelman *et al.* (1996).

The statistical analysis was performed using the multiple-marker approach with regression as described for half-sib families in Knott *et al.* (1996). Both one-QTL and two-QTL models were fitted. First, the most likely linkage phase of the gametes of grandsire was determined. Since the regression program calculates this interval by interval, the phases of grandsires were checked with the CRI-MAP (V 2·4, 1990) program which uses the information from the whole chromosome simultaneously. The probability of the son inheriting the first grandsire gamete was calculated for positions at 1 cM intervals along the chromosome. This probability was computed using the estimated recombination fraction between the two closest informative flanking markers. Each position was assessed for the existence of a QTL by regressing the trait score on the probability. The analysis was a weighted least squares where the weights were the numbers of daughters. The regression was nested within grandsires and the test statistic was computed by pooling the sums of squares due to regression over grandsires and forming the *F* ratio of the regression mean square to the residual mean square (see Vilkki *et al.* 1997 for details).

The multiple regression analysis with the one-QTL model was extended to a two-QTL model as described by Spelman *et al.* (1996). In that analysis a single-QTL model was first fitted: a test statistic was calculated for one QTL vs. none. Secondly, a test statistic was calculated for two QTLs vs. none affecting the trait under study on chromosome 6. Two QTLs were fitted simultaneously at all possible combinations at a grid of 5 cM, with a minimal distance of 10 cM between the two considered intervals. To overcome singularity problems as pointed out by Spelman *et al.* (1996), families 33090 and 36022 were excluded from the two-QTL analysis, because they lacked information at the distal end of the chromosome (see Table 1).

The empirical threshold values testing one-QTL vs. none and two-QTL vs. none were obtained by permutation. The permutation test of Churchill & Doerge (1994), an empirical approach that is capable of reflecting the characteristics of a particular experiment, was used to find threshold values for the analysis. In permutation analysis the trait scores were shuffled, and genotypes retained. The *F* ratio was calculated at each analysis point at 1 cM intervals, the highest value stored, and this procedure was repeated 10 000 times. Two kind of threshold values were calculated. First, chromosome-wise threshold values (P_{exp}) were obtained. These values were corrected to experiment-wise threshold values (P_{exp}) with the formula $P_{exp} = 1-(1-P_{chr})^3$, where 3 is the number of independent tests (number of independent traits). The number of independent traits was determined by canonical correlation. Eigen-values were computed from the correlation matrix of the studied five milk production traits. Three largest accounted for 99% of the covariation among traits, indicating that there were three independent groups amongst the analysed five traits. Genome-wise threshold values were calculated with the same formula as for correction of independent traits, except the number of bovine autosomes, 29, being the exponent.

If the statistic for two QTLs vs. none was significant it was tested whether the two QTLs explained significantly more variation than the best QTL from the single-QTL analysis. This was tested with an *F*-test on the mean squares from the two-QTL model compared to that of the best one-QTL. The improvement of the two-QTL model compared to the one-QTL model was evaluated from a standard *F* table with the number of grandsires as nominator and the total number of offspring minus three times number of grandsires as denominator.

Results

The marker informativeness for the 11 microsatellites and the casein haplotype varied strikingly. The polymorphism information content values ranged from 0·16 (marker *ILSTS87*) to 0·83 (marker *BM143*). The mean heterozygosity for all 12 markers within the 12 grandsires was 49% and the proportion of informative sons in all informative families was 60%. The numbers of informative sons for each marker and the map distances (Haldane) for chromosome 6 markers estimated from our families are presented in Table 1. The information content calculated from the variance of QTL conditional probabilities as a proportion of the variance when true descent is known is presented in Fig. 1. The order of the first three marker loci from the centromere was *ILSTS93–ILSTS90–BM1329*. This order of loci is the same as in the map of Kappes *et al.* (1997), compared the inverse order of loci *ILSTS90* and *ILSTS93* in the map

Table 1. Number of sons included in the analysis per family and number of informative sons per locus

Family	No. of sons	Marker locus										
		ILSTS93	*ILSTS90*	*BM1329*	*BM143*	*ILSTS97*	*BM4528*	*RM28*	*CASEIN*	*AFR227*	*BD7*	*BM2320*
33090	30	18	16*	21	–	–	–	–	–	–	–	–
33787	28	–	–	18	23	–	10	12	20	10	–	17
34740	59	–	30	28	47	–	–	22	32	–	32	36
34798	40	23	–	20	36	18	17	–	29	–	18*	27
34872	50	–	22	30	39	–	–	30	46	–	21	34
35076	21	13	–	14	19	–	–	12	13	–	–	13
35142	80	47	39	–	72	37	63	–	–	–	47	–
35144	29	–	–	22	28	–	27	14	–	–	17	–
36022	27	17	–	15	–	–	14	–	–	–	–	–
36378	41	33	–	27	34	–	21	16	–	–	39†	26
36386	40	21	–	–	–	18	20	–	21	–	15	–
36455	35	24	–	27	31	–	–	18	23	21	23	27
Total	480	107	196	222	329	73	172	124	184	31	212	180
Map distance (cM)			17	33	20	17	2	5	9	3	5	36

*These sons were omitted from the analysis because only one of them inherited the alternative paternal allele.
†The allele information from locus ILSTS87.

of Barendse *et al.* (1997). The map of chromosome 6 spanned 147 cM.

The linkage phases of gametes of grandsires were the same whether they were determined interval by interval with regression program or by considering the whole chromosome simultaneously with CRI-MAP. Only one discrepancy was found in one family (36378) and in one marker (*BM2320*), where equal number of sons had received recombinant or parental combination of alleles. The opposite linkage phase for this grandsire at this marker is however, very unlikely to have influence on the analysis since this was the last marker in the linkage group and no measurable variation in this family at this position was detected.

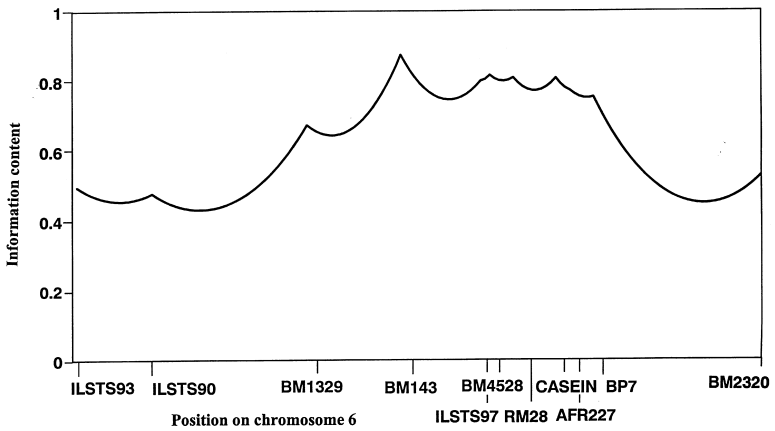

Fig. 1. Information content along chromosome six. The level was computed from the variance of QTL conditional probabilities at each cM as a proportion of the variance when true descent is known.

One-QTL model

The across-family analysis with the one-QTL model for five milk production traits revealed an effect on protein percentage and on milk yield. These effects were significant with $P_{chr} = 0{\cdot}028$ and $P_{exp} = 0{\cdot}083$ levels for protein percentage and with $P_{chr} = 0{\cdot}039$ and $P_{exp} = 0{\cdot}113$ levels for milk yield (Fig. 2). The genome-wise risk level for protein percentage was $P = 0{\cdot}52$. In addition to test statistic curves for milk yield and protein percentage, a curve for fat percentage is shown for comparison in Fig. 2. These findings were then considered more closely by looking at the individual families from the same analysis. The results within families revealed three families (out of 12) with significant effects ($P_{chr} < 0{\cdot}01$) on protein percentage (Fig. 3). The most likely positions of these effects varied within a 53-cM interval: 56 cM in family 35142, 71 cM in family 34798, and 109 cM in family 34872. For milk yield two families were identified as having significant effects: family 35142 ($P_{chr} < 0{\cdot}01$) at 70 cM, and family 34740 ($P_{chr} < 0{\cdot}05$) at 38 cM.

The effects of putative QTLs for protein percentage and for milk yield are shown in Table 2. In these families the effect caused an increase in protein percentage and at the same time a decrease in milk yield or vice versa. QTL mapping by regression tends, however, to overestimate effects of loci. Therefore the estimates should be considered with some caution.

Two-QTL model

The different locations of effects within families

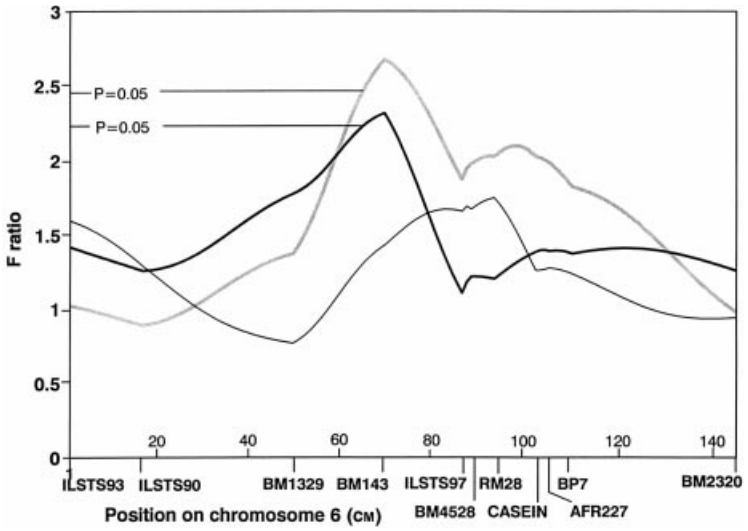

Fig. 2. Interval mapping of chromosome 6 with a one-QTL model. Test statistic curves are shown from across-family analysis for the three traits: (grey line) protein percentage; (thick line) milk yield; (thin line) fat percentage.

for protein percentage and milk yield suggested there being more than one QTL. The interval mapping with one-QTL model was therefore extended to a two-QTL analysis to locate the putative QTLs more precisely and an additional test was carried out with a two-QTL model. When testing two vs. no QTL, the test statistic exceeded the chromosome-wise risk level of 5% for protein percentage, milk yield, and fat yield. The estimated positions for these effects were 71 and 96 cM for protein percentage (Fig. 4.), 61 and 101 cM for milk yield, and 86 and 101 cM for fat yield. However, as seen for protein percentage in Fig. 4, the likelihood surface looks flat between 70 and 147 cM indicating a very faint support for the estimated positions of QTLs.

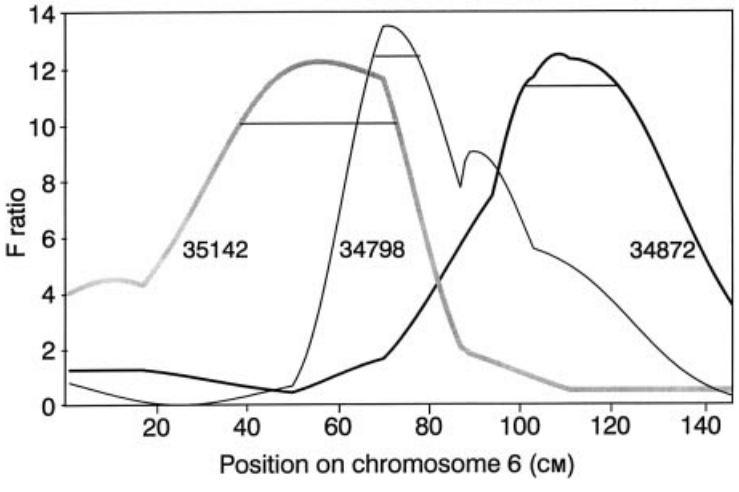

Fig. 3. Within-family results for protein percentage. The 1% chromosome-wise significance level (a line within each curve) was at *F* ratios 10·1, 12·5, and 11·4 for families 35142, 34798, and 34872, respectively.

When testing two vs. best single QTL, the effects for protein percentage and milk yield were significant at 10% risk level, but in the case of fat yield an evidence for the two QTLs was significant at 5% risk level (Table 3).

Discussion

The results of this study suggest the existence of two QTLs on cattle chromosome 6. The first putative QTL, located around marker *BM143* (at position 61–71 cM), had an effect on protein percentage and milk yield. In the within-family analysis for protein percentage the effect was, however, located at three different positions in three families. In one of these families, in family 35142, *BM1329* was not informative resulting in a 53-cM gap between informative markers. This gap probably has caused a bias in estimating the location of QTL. Moreover, the distance between position with the maximum *F*-value in this family and in family 34798 was only 15 cM and the corresponding peaks overlapped (see Fig. 3). Therefore we hypothesised that the effects in these two families could well have arisen from the same putative QTL, located near marker *BM143* (71 cM). The results from the two-QTL analysis for protein percentage, when testing two QTLs vs. none, supported the estimated location at 71 cM. However, the two families do not share common alleles at marker *BM143*, so no direct association between a marker allele and the QTL was seen. This could mean either that the QTL is not very tightly linked to marker *BM143*, or that different QTL alleles for the same locus segregate in these families. Moreover, the analysis for milk yield located an effect with the one-QTL model in the across-family and in the within-family (for family 35142) analysis at position 71 cM. The first QTL under the two-QTL model for milk yield was located at position 61 cM. Thus, the effect estimated at those positions for milk yield probably reflects the action of the same putative QTL that was identified for protein percentage at position 71 cM.

In the earlier single-marker analysis (Velmala *et al.* 1995) we found that milk yield and fat percentage were associated with casein haplotypes (in this study around 103 cM) in family 34798. In this study, the same family revealed an effect on protein percentage at position 71 cM. The single-marker analysis was therefore able to detect a QTL which was quite far (about 30 cM) from the marker, because the associated effect was so large.

The second putative QTL was localised at position 96–109 cM, around the casein complex

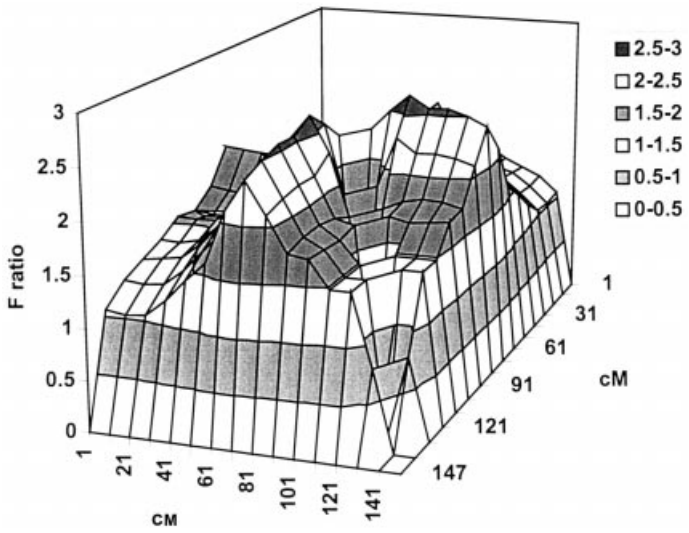

Fig. 4. Interval mapping across-families with a two-QTL model for protein percentage.

(103 cM), having effects on protein percentage, milk yield, and fat yield. Within-family analysis for protein percentage identified one family, 34872, with an effect at the map position 109 cM. In this family casein haplotypes were β-*casein* A^1–ms^5–κ-*casein B* and β-*casein* A^1–ms^4–κ-*casein E*, the former haplotype been associated with higher protein percentage compared with the latter haplotype. This was the only family in whole analysis that had these two casein haplotypes contrasted. Interestingly, no effect for milk yield in this family was observed, although the across-family analysis with the two-QTL model for milk yield identified the second QTL at position 101 cM. The second QTL under the two-QTL model for fat yield was localised at the same position (101 cM). A simple explanation why the one-QTL analysis did not detect any QTL for fat yield while the two-QTL model did, is that in the analysed families the effects were only 10–15 cM apart with plus alleles in repulsion phase for that trait.

In this study analysis with the two-QTL model gave results which supported the results from within-family analysis with the one-QTL model for protein percentage. The test of two QTLs vs. best one QTL, which was carried out when the test of two QTLs vs. none was significant has one shortcoming: If the best one QTL is a 'ghost' arising from two flanking QTLs, the variance explained by that one QTL may, depending on the distance between the loci, be as much as that by the two separate QTLs. In that case the two QTLs will not be significantly better than the 'ghost' one QTL and in that manner the test is too conservative. However, from the results of the two QTL analyses it seems that the initial one QTL was detected plus an additional QTL in another region. So none of the initial one QTLs seemed to be a 'ghost' QTL. A more conservative test for the two-QTL model against the one-QTL model would have been to use a second one-QTL model after correcting the data for the effect at the best position in the first one-QTL model and find the significant test statistic value by permutation analysis. However, since our results on the existence of two QTLs are only suggestive, we did not apply such a more stringent test procedure.

The importance of choosing the risk level for QTL studies to minimize type-I errors has recently been discussed at length. Lander & Kruglyak (1995) suggest that thresholds for linkage should always be calculated assuming a genome-wide scan, no matter what portion of the genome is effectively analysed. In this study we aimed at identifying candidate regions on chromosome 6 for a QTL effect suggested by an earlier single–marker association study (Velmala *et al.* 1995). In addition, QTLs affecting milk production traits on chromosome six have been previously reported by others (Georges *et al.* 1995; Kühn *et al.* 1996; Spelman *et al.* 1996; Zhang *et al.* 1998). The available information encouraged us to report our findings both with chromosome-wise risk levels and with the risk levels obtained after a Bonferroni correction

Table 2. Within-family QTL substitution effects and their standard errors for protein percentage and milk yield. The standard deviation of breeding value estimates in the present bull population is 0·14% for protein percentage and 350 kg for milk yield

Trait	Family	QTL effect	Position (cM)
Prot percentage	34798	0·14 (0·04)%	71
	34872	0·11 (0·03)%	109
	35142	0·09 (0·03)%	56
Milk yield	34740	350 (122) kg	38
	35142	242 (70) kg	70

Table 3. Results from the analysis with a two-QTL model

	Protein percentage	Milk yield	Fat yield
Positions	71, 96	61, 101	86, 101
F ratio for two vs. no QTL	2·68	2·26	2·02
Threshold level of 1%*	2·38	2·29	2·29
Threshold level of 5%*	2·04	1·96	1·96
F ratio for two vs. best single QTL	1·82	1·75	1·87
Threshold level of 5%†	1·85	1·85	1·85
Threshold level of 10%†	1·62	1·62	1·62

*Chromosome-wise levels obtained by permutation.
†Tabulated values.

for three independent tests. Genome-wise risk levels were also calculated for comparison.

Within studies reporting a QTL on chromosome 6, Georges *et al.* (1995) studied one interval some 30 cM long and found a QTL about 20 cM away from the casein loci towards the centromere affecting protein percentage, fat percentage and milk yield in one family. Kühn *et al.* (1996) found significant differences in the protein percentage among sons inheriting two alternative paternal alleles for marker *BM143* in one German Holstein–Friesian family. Spelman *et al.* (1996) screened chromosome 6 for five milk production traits in Dutch Holstein–Friesian cattle and identified a QTL for protein percentage close to marker *BM143*. Recently a large QTL study conducted by Zhang *et al.* (1998) confirmed the findings by Georges *et al.* (1995) and Spelman *et al.* (1996) for chromosome 6. These findings may all reflect the presence of the same QTL due to common ancestors in the American and European black-and-white dairy cattle populations. Our study covers a completely distinct breed, the Ayrshire. Nevertheless, the effect for protein percentage found near marker position *BM143* may be due to the same QTL as in Holstein–Friesian populations. Also the effect of this putative QTL in Finnish Ayrshire seems to be similar to that of the QTL in US Holsteins (Georges *et al.* 1995) in the sense that it affects milk yield and composition in opposite ways.

It has been hypothesised by Georges & Andersson (1996) that different breeds may share identity-by-descent (IBD) QTL alleles, which have been maintained through similar selection goals. Recently an IBD mapping strategy has been proposed for genetic fine-mapping of the QTL affecting milk production traits on bovine chromosome 20 (Arranz *et al.* 1998). Similarly, the IBD approach could be used here in interbreed comparisons to assign the QTL on chromosome 6 to IBD chromosomal segments, and thus to enable this QTL to be mapped more precisely.

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