Population-Wide Analysis of a QTL Affecting Milk-Fat Production in the Israeli Holstein Population

J. I. Weller*, M. Golik*, E. Seroussi*, E. Ezra†, and M. Ron*

*Institute of Animal Sciences,
Agricultural Research Organization,
The Volcani Center, Bet Dagan, Israel 50250

†Israel Cattle Breeders Association
Caesaria Industrial Park, Caesaria, Israel 38900

ABSTRACT

A population-wide linkage disequilibrium on bovine chromosome 14 between microsatellite ILSTS039 and *DGAT1*, a putative quantitative trait locus affecting milk production traits, was found in the Israeli Holstein population. A total of 394 bulls were genotyped for both *DGAT1* and ILSTS039, and 1747 cows were genotyped for ILSTS039. The ILSTS039 allele termed “225,” and the *DGAT1* K allele (substitution of a lysine residue with alanine), were associated with decreased milk production, and increased fat production and fat and protein percent. The number of 225 ILSTS039 and K *DGAT1* alleles per individual were the same for 80% of the bulls genotyped. From the effects associated with cows homozygous for the 225 allele, the effect of the quantitative trait locus appears to be approximately codominant. The substitution effect was 0.16% fat. Genotype probabilities for the quantitative gene were determined for the entire Israeli Holstein milk-recorded population, including 507,725 cows and 1442 bulls, using segregation analysis. Overall frequency of the allele that increased fat percent was 8.9% in cows and 15.5% in bulls. The frequency of this allele decreased from 1981 until 1990, from 15 to 5%, and since has increased to 10%. The effects estimated on the population-wide analyses of both cows and bulls were similar to the effect associated with *DGAT1* in the daughters of genotyped bulls. Modified animal model evaluations were computed for the entire population with the effect of this gene included in the model. The correlations between the modified and standard animal model evaluations for all traits were > 0.99.

(Key words: cattle microsatellite, quantitative trait loci, bovine chromosome 14, marker-assisted selection)

Abbreviation key: BV = breeding value, DYD = daughter yield deviation, IBD = identical by descent, LD = linkage disequilibrium.

INTRODUCTION

Many studies have shown that individual QTL can be detected and mapped in commercial dairy cattle populations with the aid of genetic markers by application of daughter or granddaughter designs (Weller et al., 1990). Several studies beginning with Ron et al. (1998) found a major QTL associated with milk-fat concentration segregating in the Israeli and US Holstein dairy cattle populations near the centromeric end of bovine chromosome 14. The locus was also found to be segregating in the Dutch Holstein population (Coppieters et al., 1998) and German dairy cattle population (Looft et al., 2001). Riquet et al. (1999) used identical by descent (IBD) mapping to localize this QTL to a region near the centromeric end of the chromosome, including CSSM66, but excluding marker ILSTS039 at the extreme end of the chromosome. Heyen et al. (1999) used interval mapping to localize the QTL to the ILSTS039-CSSM66 interval, but the likelihood maximum was closer to ILSTS039. Recently, Grisart et al. (2002) identified a polymorphism in exon VIII of the gene acylCoA:diacylglycerol acyltransferase (*DGAT1*), which results in the substitution of a lysine residue with alanine. *DGAT1* is located in the confidence interval for the QTL on BTA14, and a knockout mutation completely inhibited lactation in mice. Grisart et al. (2002) genotyped 1818 Dutch Holstein sires and 529 New Zealand Holstein cows for this polymorphism. In both populations the *DGAT1* allele with a lysine residue (denoted K), as opposed to alanine residue (denoted A), was associated with increased fat yield and fat and protein percent, and decreased milk and protein production. All these effects correspond to the QTL effects observed in daughter and granddaughter designs. However, the magnitudes of effects were not similar in the two populations. For the yield traits the absolute values of the substitution effects were greater in the Dutch population, while the effects on the percent traits were similar.

Kinghorn and Kerr (1995) and Israel and Weller (1998) proposed a method to obtain unbiased estimates of QTL effects via a modified animal model, even though

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Corresponding author: J. I. Weller; e-mail: weller@agri.huji.ac.il.

only a small fraction of the population was genotyped, provided that QTL genotype probabilities can be derived for all animals. Kerr and Kinghorn (1996) derived an algorithm to estimate genotype probabilities for all animals in a population, based on a sample of individuals with known genotypes. Israel and Weller (2002) applied this method on simulated data to obtain unbiased estimates of QTL effects via a modified animal model analysis.

Meuwissen and Goddard (2002) proposed that population-wide linkage disequilibrium (LD) could be used to fine map QTL. Looft et al. (2001) presented evidence for population-wide LD in the German dairy cattle population between the QTL and a polymorphism in an EST, KIEL_E8, which is very tightly linked to ILSTS039.

This study reports on the effect of the QTL on BTA14 in the Israeli-Holstein population and the genetic trend for the allelic frequencies. Various analysis methods are compared, and genetic evaluations including the QTL effect were computed based on the method of Israel and Weller (2002). In addition we report on a population-wide LD in the Israeli population between the QTL on chromosome 14 and microsatellite ILSTS039. The allele termed "225," based on the length of the PCR product, was associated with increased fat percent in all heterozygous families in both the US and Israeli Holstein populations. Allele 225 was the shortest of eight alleles observed in the Israeli population. This LD was verified by genotyping large samples of Israeli cows and bulls. Partial LD was also found in the US population.

MATERIALS AND METHODS

Population Sample

Blood samples were collected from over 13,000 Israeli-Holstein cows, daughters of 11 sires from 233 herds. Semen samples were collected from the 11 sires. Cows ($n = 6047$) were genotyped for at least five microsatellites to confirm paternity. Cows that did not inherit either paternal allele for at least two loci were considered to be not daughters of the sire listed, and were therefore deleted from further analysis. Cows without genetic evaluations for all five production traits; milk, fat, and protein production, and fat and protein percentage were also deleted from the analysis.

Ten of the 11 sires were genotyped for ILSTS039, and seven sires were heterozygous. The daughters of the heterozygous sires were genotyped for this marker. (The final sire did not have a sufficient sample of daughters at the time of the ILSTS039 genotyping.) There were 1747 cows with genetic evaluations for production traits, and valid genotypes for ILSTS039. Eighty-six

daughters of sire 3241, which was heterozygous for both *DGAT1* and the QTL affecting fat percent, as determined by the daughter design analysis, were also genotyped for *DGAT1*. Artificial insemination sires ($n = 424$) with genetic evaluations were genotyped for the *DGAT1* polymorphism. Of these, 394 were also genotyped for ILSTS039. Genotypes were determined for most Israeli AI sires born since 1982, and all but one of the sires born since 1982 that were returned to general service.

Genotyping Methods

DNA from frozen blood or semen was extracted by the salting out procedure (Ma et al., 1996). DNA was diluted to 7 ng/ μ l, and 5 μ l was aliquoted to 96-well and 384-well plates using Hydra robotic system (Robbins Scientific, www.robsci.com). DNA in plates was dried and stored at room temperature. The PCR protocols for DNA isolated from semen and blood cells were as described by Ron et al. (1995) using a DNA engine thermocycler (MJ Research, Inc., www.mjr.com).

The K (lysine) allele-specific primer (CAGCTTTGGCAGGTAAGAA) for *DGAT1* was labeled with FAM (blue) fluorophore and the A (alanine) allele-specific primer (CAGCTTTGGCAGGTAAGGC) was labeled with HEX (yellow) fluorophore. These forward primers were used in separate amplification reactions with one reverse primer (TAGGTCAGGTTGTCGGGGTA). Ten-microliter PCR reactions consisted of 1 μ l of PCR Buffer (JMR-435, JMR-Holdings, UK), 5 pmol of each primer, 1 μ l of dNTP, 0.24 U *Taq* polymerase (JMR-801, JMR-Holdings), and 35 ng of whole genomic template DNA. Cycling conditions were as follows: initial denaturation of 94°C for 3 min, followed by 30 cycles of 92°C for 40 s, variable annealing (at 60°C for the K allele and at 66°C for the A allele) for 40 s, 72°C for 1 min, and a final single extension step of 72°C for 10 min. After PCR, the two allele-specific reactions of each individual were mixed and 0.6 μ l were added to 0.6 μ l of loading buffer (75% formamide/dye, 25% MapMarker Low, BioVentures, Murfreesboro, TN). These samples were denatured at 92°C for 3 min and cooled on ice.

PCR reactions were run on the ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA). Automated fragment analysis, size calling, and binning were then used by GeneScan (Version 3.1) and Genotyper (Version 2.0) genetic software (Applied Biosystems) to identify the alleles of each locus.

Phenotypic Records and Statistical Methods

The official Israeli Holstein genetic evaluations are computed twice yearly at the Agricultural Research Organization. Three hundred and five-day milk, fat, and

Table 1. Means and standard deviations for the genetic evaluations of the 1747 cows genotyped.

Trait	Mean	Standard deviation
Milk (kgs)	234	509
Fat (kgs)	7.4	14.5
Protein (kgs)	1.9	11.3
% fat	0.002	0.180
% protein	-0.045	0.095

protein production, preadjusted for calving age and month and days open, were analyzed by a repeatability animal model (Weller et al., 1994). First through fifth parity production records of all animals with valid records for all three traits, and first-parity calving dates since 1985 were included in the analysis. All known relationships among animals were included via the numerator relationship matrix. Later parity records were included only if there were valid records for all previous parities. The additive genetic and permanent environmental variance components were each assumed to be 0.25 of the total variance for all three traits. The variance component values were verified by REML on a subset of the data. The REML variance component estimates were close to the values used in the animal model analyses for all three traits. Genetic evaluations for fat percentage for each cow were derived as follows:

$$BV_{FP} = [(BV_F + M_F)/(BV_M + M_M)] - M_{FP},$$

where BV_{FP} , BV_F , BV_M are the cow's estimated breeding values for fat percentage, fat yield, and milk; and M_F , M_M , and M_{FP} are mean adjusted first-parity fat yield, milk, and fat percentage of cows born in 1995. Genetic evaluations for protein percentage are computed similarly, with protein yield and percentage, instead of fat yield and percentage. The February, 2002, evaluations were analyzed. Means, standard deviations, and minimum and maximum values of genetic evaluations of the cows genotyped for the five traits analyzed are given in Table 1, and the correlations among the evaluations are given in Table 2.

Preliminary QTL analysis for ILSTS039 was by the following linear model for all five traits:

Table 2. Correlations among the genetic evaluations of the 1747 cows genotyped.

Trait	Milk	Fat	Protein	% fat	% protein
Milk (kgs)	1	0.21	0.72	-0.68	-0.66
Fat (kgs)		1	0.24	0.57	-0.04
Protein (kgs)			1	-0.43	0.04
% fat				1	0.52
% protein					1

$$BV_{ijk} = S_i + M_{ij} + e_{ijk},$$

where BV_{ijk} is the estimated cow breeding value of cow k , daughter of sire i , that received paternal marker allele j ; S_i is the effect of sire i on the trait; M_{ij} is the effect of paternal allele j of sire i on the trait, and e_{ijk} is the random residual associated with each record. If the daughter had the same genotype as her sire, paternal allele origin could not be determined, and these cows were deleted from the analysis. A significant paternal allele effect is indicative of a segregating QTL linked to the genetic marker. Significance of the paternal allele contrast within each family was determined by a t -test with residual variance computed across all cows. A sire was considered heterozygous for the QTL if the t probability was less than 0.01.

In addition the effect of marker ILSTS039 on the cows' breeding values was analyzed by the following model:

$$BV_{ijk} = S_i + M_j + e_{ijk},$$

where M_j is the effect of the number of 225 alleles for each cow, which could have values of zero, 1, or 2, and the other terms are as defined previously. This model was analyzed with M as a regression effect, and M as a class effect (class effect, model 1). In addition the model was also analyzed with M as a class effect, but with the cows with one 225 allele divided into two groups; those that were daughters of sires that had the 225 allele, and those cows that were daughters of sires that did not have the 225 allele (class effect, model 2).

The sire breeding values for all five traits were analyzed by the following model:

$$BVS_i = BY + BY^2 + D + e_i,$$

where BVS_i is the breeding value of sire i , BY and BY^2 are the linear and quadratic regression effects of the sires' birth year, and D is the regression effect of the number of *DGAT1* K alleles for each sire. The BY and BY^2 effects were included to account for changes in the frequency of the K allele over time. The sire breeding values were also analyzed by the following model

$$BVS_i = BY + BY^2 + D + M + e_i,$$

where M is the regression effect of the number of 225 alleles for ILSTS039, and the other terms are as defined previously.

Only a small fraction of the cow population was genotyped for either ILSTS039 or *DGAT1*. Genotype probabilities for the QTL were determined for the entire Israeli Holstein milk-recorded population, including

Table 3. The allele substitution effects associated with ILSTS039, their standard errors, and the t-test probabilities by the daughter design analysis.

Trait	Sire	
	2283	3241
Milk (kgs)	-193 ± 54***	-106 ± 48*
Fat (kgs)	4.5 ± 1.9*	7.1 ± 1.7****
Protein (kgs)	-0.6 ± 1.4	-1.1 ± 1.2
% fat	0.102 ± 0.017****	0.100 ± 0.015****
% protein	0.048 ± 0.009****	0.019 ± 0.008*
Number of informative daughters	202	261

* $P \leq 0.05$.*** $P \leq 0.001$.**** $P \leq 0.0001$.

507,725 cows and 1442 bulls, using the segregation analysis algorithm of Kerr and Kinghorn (1996). In this analysis complete linkage was assumed between the QTL allele that increased fat concentration and the K allele of *DGAT1* for bulls, and between the QTL and the 225 allele of ILSTS039 for cows that were genotyped for this marker. The number of animals analyzed by the segregation analysis algorithm was reduced to 33,292 by four “pruning” steps. At each step, cows that were not genotyped, and were not listed as dams of cows remaining in the data file were deleted. The pruning did not affect the segregating analysis, because these cows by definition include no information with respect to the allelic frequencies. The algorithm requires an estimate of the allelic frequencies in the base population. The initial estimate was derived from the frequencies of the genotyped bulls. After application of the algorithm this estimate was revised, based on the allelic frequencies of all animals with unknown parents. The segregation analysis algorithm was rerun with the updated base population allelic frequencies until convergence for the base population allelic frequencies was obtained. The genotype probabilities for the “pruned” cows were then regenerated from the genotype probabilities of their parents, assuming random distribution of alleles. For cows with either one or two unknown parents, the allelic frequencies of the base population were used for the unknown parent.

The estimated allelic frequencies as a function of birth year were computed for the entire population of bulls and cows. Modified animal model evaluations were computed for the entire population for milk, fat, and protein production including the QTL as a fixed effect as described by Israel and Weller (2002). The QTL effect was assumed to be additive. Therefore only the frequency of rare QTL allele was included in the model. Modified genetic evaluations, consisting of the sum of the polygenic and QTL effects for each individual were compared to the standard genetic evaluations de-

rived from a standard animal model. In addition, the QTL effect was estimated separately for all bulls and cows with evaluations by the following model:

$$BV_i = BY + BY^2 + Q + e_i,$$

where Q is the regression effect of the expected number of *DGAT1* K alleles for animal i, and the other terms are as defined previously. Cows born before 1981 and bulls born before 1961 were deleted. Bulls with reliabilities for production traits less than 0.5 were also deleted from this analysis.

RESULTS

Table 3 includes the effects of ILSTS093 from the daughter design on production traits for the two families with significant effects. Significant effects were found for milk and fat production, and fat and protein percent for both sires. The effect on fat percent was highly significant ($P < 0.0001$) for both sires. Significant effects were not found for protein production. In both sires, allele 225 was associated with increased fat and decreased milk production. None of the other sires analyzed had allele 225. Both of the sires that were heterozygous for the QTL were also heterozygous for *DGAT1*. The five other sires analyzed in the daughter design were all homozygous for the A allele of *DGAT1*.

Effects of allele 225 on the cows' genetic evaluations from all seven families analyzed and the standard errors for all five traits for all three-analysis models are given in Table 4. The frequency of the 225 allele in the sample of cows genotyped was 12%. The effect of allele 225 was significant for all five traits in the regression model analysis of cow breeding values, supporting the hypothesis of population-wide LD between the 225 allele and the QTL. Except for the effect on protein, the effects of allele 225 were slightly less than the mean of the effects found in the daughter design analysis for

Table 4. Effects of allele 225 on the cows' genetic evaluations and standard errors for all five traits for all three analysis models¹.

		Class effect, Model 1		Class effect, Model 2		
Model						
No. 225 alleles	Regression	1	2	1 _d ²	2	1 _{nd} ³
Trait:						
Milk (kgs)	-127 ± 23****	-127 ± 24****	-302 ± 91****	-127 ± 32***	-302 ± 91**	-127 ± 38***
Fat (kgs)	3.4 ± 0.78****	3.4 ± 0.8****	6.4 ± 3.2*	4.5 ± 1.1****	7.0 ± 3.2*	1.9 ± 1.3
Protein (kgs)	-2.0 ± 0.57***	-2.0 ± 0.6**	-4.0 ± 2.3	-1.4 ± 0.8	-3.7 ± 2.3	-2.9 ± 1.0**
% fat	0.072 ± 0.007****	0.070 ± 0.008****	0.153 ± 0.029****	0.082 ± 0.10****	0.159 ± 0.029****	0.054 ± 0.012****
% protein	0.018 ± 0.004****	0.017 ± 0.004****	0.047 ± 0.016**	0.023 ± 0.006****	0.050 ± 0.016**	0.008 ± 0.007
Number of cows	1749	384	18	276	18	108

¹Effects in the class effect models, significance levels, and standard errors are given relative to cows with zero 225 alleles. There were 1347 cows with zero 225 alleles.

²Cows with one 225 allele, and daughters of the two sires heterozygous for this allele.

³Cows with one 225 allele, but not daughters of the two sires heterozygous for this allele.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

**** $P \leq 0.0001$.

the two families with significant contrasts. However, from the standard errors it is clear that differences were not significant.

Effects in the class effect models are given relative to the class with zero 225 alleles. In the class effect models, the effect of a single 225 allele was generally similar to the effects in the regression model, and the effects of two 225 alleles were approximately equal to twice the effect of a single 225 allele. The latter results give further support to population-wide LD, because one of the 225 alleles of these cows must have come from the cows' dams, and is not due to the specific linkage phase relationships in the two heterozygous sires. These results also indicate that the effect of the QTL is approximately additive, as also determined by Grisart et al. (2002). However, there were only 18 cows that were homozygous for the 225 allele. Furthermore, if LD is incomplete, then the effect associated with the 225 allele homozygotes will be greater than heterozygotes, even if there is complete dominance at the QTL. Thus, the conclusion of additivity must still be considered tentative. Further support for population-wide LD is presented by the results of class effect model 2. The effects obtained for cows that received a 225 allele, but were not daughters of either sire 2283 or 3241, were similar to the effects obtained for those cows that were daughters of these two sires and received a single 225 allele. The effects obtained for the cows that were not daughters of the heterozygous sires were equal for milk, slightly greater for protein, but less for the other traits. None of the differences between the effects for cows with one 225 allele that were daughters of the two sires segregating for the QTL, and cows with one 225 allele that were daughters of the other sires were significant, except for the effect on fat yield.

Of 86 daughters of sire 3241 that were genotyped for both loci, there were only seven cows for which the number of K alleles for *DGAT1* was not equal to the number of ILSTS039 225 alleles, 8.1%. However, since the dams were not genotyped, paternal allele origin can only be determined if the daughter genotype is different from her sire. There were 39 cows that met this requirement for both loci. Of these, only one cow was a recombinant. This corresponds to a recombination frequency of 2.5% between ILSTS039 and *DGAT1*.

The joint frequencies of the *DGAT1* and ILSTS039 genotypes for the genotyped bulls are given in Table 5. Again, with respect to ILSTS039, only the number of 225 alleles is considered. Although all nine possible genotypes were found, the allelic frequencies for the two loci were highly correlated. Of the 394 bulls genotyped there were only 81 in which the number of 225 ILSTS039 alleles was not equal to the number of *DGAT1* K alleles (20%). The χ^2 value for the test of independent assortment was 159. With four degrees of freedom this value is highly significant by any criterion. Thus there is strong LD between these two loci. The frequency of the 225 allele among the bulls genotyped was 17.1%, which is somewhat higher than among the

Table 5. Frequency table of bull genotypes for *DGAT1* and ILSTS039.

Number of "225" alleles for ILSTS039	Number of "K" alleles for <i>DGAT1</i>			
	0	1	2	Total
0	248	22	1	271
1	45	62	4	111
2	2	7	3	12
Total	295	91	8	394

Table 6. QTL substitution effects and their standard errors on the bull's genetic evaluations for bulls that were genotyped for *DGAT1*, and for all bulls and cows based on the algorithm of Kerr and Kinghorn (1996).

Trait	Dependent variable		
	Bulls with genotypes	All bulls ¹	All cows ²
Milk (kgs)	-306 ± 62****	-258 ± 48****	-443 ± 3
Fat (kgs)	7.4 ± 2.1***	8.7 ± 1.6****	2.8 ± 0.1****
Protein (kgs)	-3.1 ± 1.5*	0.8 ± 1.2	-7.4 ± 0.1****
% fat	0.164 ± 0.021****	0.162 ± 0.016****	0.0160 ± 0.001****
% protein	0.057 ± 0.012****	0.081 ± 0.009****	0.054 ± 0.001****
Number of animals	416	1038	491,613

¹Born since 1961 with reliability greater than 0.5.²Born since 1981.* $P \leq 0.05$.*** $P \leq 0.001$.**** $P \leq 0.0001$.

cows. The frequency of the K allele of *DGAT1* was 13.6%.

The effects of *DGAT1* genotype in the analyses of the sire evaluations are given in Table 6. A total of 416 bulls born since 1960 were genotyped, and the effects on these bulls are given in the first column of the table. The effects were significant for all five traits. In the model that included both the effects of *DGAT1* and ILSTS039, only the effect of *DGAT1* was significant by the type III sum of squares (SAS, 1988), which corrects for all other effects in the model. This gives further confirmation that *DGAT1* is in fact the QTL. It is expected that the QTL effects in the sire analyses should be greater than the effects obtained in the cow analyses, because *DGAT1*, the actual QTL, was genotyped for the bulls, while ILSTS039 was genotyped for the cows; and the cow evaluations are more highly regressed (Israel and Weller, 1998). The difference should be greater for the production traits, which have lower heritabilities than the concentration traits, and this was generally the case.

Similar results for the effect of the *DGAT1* polymorphism were obtained for the regression on the expected sire genotypes, including bulls that were not genotyped with reliability greater than 0.5. The regression effects for all cows included in the animal model analysis born since 1981 are also listed. Effects for cows were greater for milk, protein, and protein percent, but less for fat yield. The effect on fat percent, 0.16%, was nearly the same for all three analyses.

Expected allelic frequencies for the QTL for the entire cow and bull populations by birth year are given in Table 7. Overall, the frequency of the rare QTL allele was nearly double in the bull population as compared to the cows. The frequency for the allele that increases fat percent was lowest for cows born in 1991, and has since then increased from 0.053 to 0.095. Similar trends

are evident in the sire population. The frequency means of cows by birth year are plotted in Figure 1. For comparison, the estimated animal model BV means for fat and protein percent are also plotted. As can be seen,

Table 7. Estimated frequencies for the K allele of *DGAT1* in the total population of bulls and cows by birth year.

Birth year	Cows		Bulls	
	Number	Allele frequency	Number	Allele frequency
1968			7	0.147
1969			8	0.084
1970			7	0.198
1971			10	0.165
1972			15	0.110
1973			8	0.287
1974			15	0.214
1975			14	0.120
1976			23	0.116
1977			32	0.153
1978			34	0.193
1979			50	0.156
1980			42	0.164
1981	9621	0.152	38	0.150
1982	12,571	0.125	65	0.133
1983	16,060	0.091	24	0.081
1984	20,098	0.095	45	0.084
1985	22,471	0.127	44	0.188
1986	26,165	0.118	43	0.200
1987	26,394	0.104	44	0.195
1988	27,245	0.072	53	0.085
1989	27,526	0.067	41	0.105
1990	28,812	0.054	39	0.197
1991	30,277	0.053	48	0.197
1992	30,896	0.073	45	0.142
1993	33,546	0.074	57	0.115
1994	31,402	0.086	47	0.189
1995	31,428	0.099	38	0.163
1996	30,149	0.100	55	0.208
1997	30,244	0.097	26	0.188
1998	30,482	0.092		
1999	26,090	0.095		
Total	491,638	0.089	1038	0.155

Figure 1. Mean frequencies of the K allele of *DGAT1* in the cow population by birth year, and mean estimated breeding values for fat and protein percent. —, mean frequency for the K allele; ■—■, mean estimated breeding value, fat percent; ▲—▲, mean estimated breeding value, protein percent.

all three graphs show a general decrease until 1990, and then increase until 1999. These trends correspond to the change in the Israeli breeding index, which was based chiefly on milk production until 1990. Since then the index has been based chiefly on protein with a negative weight for milk yield.

The substitution effects of the QTL derived from the modified animal model analysis after 1000 iterations are given in Table 8. Effects are smaller than those obtained in the regression models in Table 6, except for the effect of fat in the analysis of all cows. The progression of the estimates of the QTL effect as a function of iteration number up to 500 iterations are given in Figure 2. In the first iterations, the effect for milk was -200 kg, and then became positive before converging to the relatively small negative value of -28. The effect for fat increased to about 8 kg, before converging to 3.4 kg. Correlations for genetic evaluations of cows derived by the standard and modified animal models including the QTL effect were all greater than 0.99 for all five milk production traits, and are therefore not presented.

DISCUSSION

The effects obtained for the QTL on BTA14 for fat and protein percent were similar to those obtained by

Table 8. Substitution effects for the K allele of *DGAT1* from the animal model evaluations.

Trait	Substitution effects
Milk (kgs)	-28
Fat (kgs)	3.4
Protein (kgs)	-0.2
% fat	0.040
% protein	0.006

Figure 2. Trends of the estimates for the *DGAT1* substitution effect by iteration. —, milk; ---, fat; —▲—, protein.

Heyen et al. (1999) in the granddaughter design analysis of the US population. In the current study, the effect observed for milk production was greater, while the effect on fat production was lower. The effect on milk production was only marginally significant in the previous study. In the US population, the two sires heterozygous for this QTL were also heterozygous for allele 225, and this allele was associated with increased fat percentage. However, two other US sires included in the granddaughter analysis were also heterozygous for the 225 allele, even though they were apparently homozygous for the QTL. Thus within the US population LD is much lower. Frequency of the 225 allele in the US Holstein population is also much higher than the Israeli population. Based on the genotypes of a sample of 90 sons from two US families (Heyen et al., 1999), the frequency of the 225 allele among the maternal alleles was 48%.

The pedigrees of the two heterozygous Israeli bulls analyzed that were heterozygous for the QTL were traced back three generations for all ancestors, and no common ancestors were found. Similarly neither of these bulls is known to be related to the two US bulls that were heterozygous for this QTL. Thus, if these four bulls received an IBD segment from a common ancestor, this ancestor must be several generations removed, and the common IBD segment is probably quite small. Farnir et al. (2002) found extensive genome-wide linkage disequilibrium in cattle for genetic markers. Thus, it is not surprising that this should be the case for the approximately 1 cM interval between ILSTS039 and *DGAT1* in the Israeli Holstein population.

Nearly all previous published QTL analyses in dairy cattle have been based on either daughter or granddaughter designs. In either case only the additive QTL effect can be detected. This is the first dairy cattle QTL for which it was also possible to estimate dominance.

The results from the analysis of the cow breeding values indicate that the QTL effect is in fact approximately codominant, similar to the results of Grisart et al. (2002). In plants, QTL with codominance, partial dominance, complete dominance, and overdominance have all been reported (e.g., Weller et al., 1988).

Two out of seven sires analyzed were segregating for this QTL in the Israeli population. This corresponds to an allelic frequency of 0.17 for the rare allele, which is close to the observed frequency of 0.15 in the entire sire population. Assuming that only two QTL alleles are segregating in the population, the variance associated with this QTL in the population can be estimated as $2p(1-p)a^2$, where p is the frequency of one of the alleles, and a is the additive effect (Weller, 2001). Using the allelic frequency estimate from the sample of genotyped cows of 10%, and the estimate of the additive effect of 0.16% fat from Tables 4, 5, and 6 gives a variance estimate of 0.0046 for this trait. The genetic variance for fat percent in the Israeli Holstein population, as determined by a REML analysis of first parity lactations, was approximately equal to 0.056. Thus, even though the additive effect of this QTL is equal to 0.68 genetic standard deviations, the QTL accounts for only about 8% of the genetic variance for fat percent.

It is therefore possible that other QTL of similar magnitude are also segregating in commercial populations. In fact, a QTL of similar effect on percent fat was also found on chromosome 6 (Ron et al., 2001). The effects observed for this locus in a daughter design were approximately 0.08% fat, and 0.065% protein. Thus this locus has a slightly smaller effect on percent fat, but a much greater effect on percent protein, relative to the genetic standard deviations for each trait.

As demonstrated by the genetic trend for this locus, selection for the current Israeli breeding index will probably continue to increase the rare allele, and therefore increase the genetic variance due to this locus. Thus, contrary to the prediction of the infinitesimal model, with segregating QTL, selection can sometimes lead to an increase in the genetic variance of the population. de Koning and Weller (1994) observed this result on simulated data.

The small effects for the QTL obtained by the modified animal model analyses were somewhat surprising. Similar results were obtained in an analysis that assumed a smaller polygenic variance, and in an analysis that did not assume additivity (data not shown). Israel and Weller (1998, 2002) found on simulated data that results obtained by this method were unbiased, while estimates derived from daughter yield deviations (DYD) or genetic evaluations underestimated the simulated effects. However, in their simulations, at least 25% of the population was actually genotyped, and the

frequency of the rare allele was no less than 0.2. Furthermore, only two or three generations were simulated, while the current analysis included close to eight generations. Finally, in the modified animal model analysis, complete linkage was assumed between the QTL and ILSTS039 for cows that were genotyped, even though this is not the case. Kinghorn and Kerr (1995) also obtained unbiased estimates of the QTL effect on simulated data, but did not include a polygenic effect in either the simulation or analysis models.

The regression models did not include the effect of relationships, which might upwardly bias the estimates. In any event, the effect of the QTL and the relationship matrix would be highly confounded in the current analysis. These two factors can only be distinguished by animals with similar pedigree but with different genotypes. This was the case for some of the cows genotyped, but was rarely the case for the bulls. In the relatively small Israeli Holstein population, there are no large half-sib families of bulls with genetic evaluations. Grisart et al. (2002) analyzed the bull DYD by a regression model that included the relationships among these animals. However, they assume equal residual variance among DYD, which is clearly not the case. Furthermore, properties of statistical models based on analysis of DYD, which are derived from animal model analyses, have not been investigated in detail. Israel and Weller (1998) found that QTL estimates derived from analysis of DYD are biased.

Mackinnon and Georges (1998) proposed marker-assisted selection based on preselection of young sires based on their genotypes for QTL. The disadvantage of this method is that individuals with superior overall genetic value are culled if they do not have the desired genotype for specific QTL under control. Israel and Weller (1998, 2002) proposed that QTL effects could be incorporated into animal model analyses even if only a small fraction of the population is actually genotyped. Using this method it should be possible to correctly rank all animal, including information on known QTL. This is the first application of their method to an actual QTL. Genetic evaluations derived by this method were nearly identical to the standard animal model evaluations. Thus, in this specific example, the gain obtained by marker-assisted selection would be minimal. However, there is also no potential loss that could be obtained by alternate MAS proposals (Weller, 2001).

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