

Blood Groups and Milk and Type Traits in Dairy Cattle: After Forty Years of Research

J. L. ROCHA,* J. O. SANDERS,* D. M. CHERBONNIER,*
T. J. LAWLOR,[†] and J. F. TAYLOR*,¹

*Department of Animal Science, Texas A&M University, College Station 77843

[†]Holstein Association, Brattleboro, VT 05301

ABSTRACT

This study addresses the utility of 11 blood groups as selection aids in Holstein breeding schemes and considers issues inherent to the approach of resolving quantitative variation into components that are due to quantitative trait loci. The data consisted of predicted transmitting abilities of 22,614 bulls, first lactation information on 1,924,171 cows, and type scores on 447,800 cows. Linear models were fitted under male half-sib designs, female half-sib designs, and granddaughter designs as well as under the assumption of direct effects of the markers. The evolution of allele frequencies through time was determined, and previous research results were synthesized according to criteria of consistency of biological significance. The inconsistency of results across studies and analytical designs alludes to the importance of the intrinsic nonadditivity of genetic and biological phenomena to quantitative trait locus detection and marker-assisted selection. In our analyses, three associations met the criteria of consistency—a C blood group effect on rump angle, an L effect on milk yield and composition traits, and an S effect on milk fat yield. The M locus appears to be directly associated with effects on milk and protein yields. An enhanced understanding of the biochemical and physiological bases of quantitative genetics should be a long-term objective of this type of genetic analysis.

(**Key words:** blood groups, milk and type traits, quantitative trait loci, marker-assisted selection)

Abbreviation key: BoLA = bovine lymphocyte antigen, HA = Holstein Association, MAS = marker-assisted selection, ME = mature equivalent, QTL = quantitative trait locus, σ_a = additive genetic standard deviation, σ_p = phenotypic standard deviation.

INTRODUCTION

Several classes of ubiquitous DNA polymorphisms that are likely to saturate the genomes of livestock species have recently been developed (43, 53), which has revived interest in theoretical aspects for the detection and utilization of quantitative trait loci [QTL (17)] in animal, human, and plant genetics. Preliminary screenings for QTL detection have also been recently reported [for recent reviews (33, 46)].

However, this topic is not new. Rocha (33) reviewed over 60 fundamental studies that were published between 1918 and 1982. Considering only associations between those blood group polymorphisms and the milk and type traits available for this study, 69 publications reported 3664 statistical tests, of which 301 were significant (33). The wealth of information available since 1951 (12), the renewed interest in the utilization of genetic markers in animal breeding, the progress made in recent years with regard to statistical tools and theoretical methods to support this type of genetic analysis, and the existence of an extensive data set provided by the Holstein Association (HA) that includes records since the 1940s were all factors that coalesced to motivate this study.

The objectives were to bring into perspective over 40 yr of research on associations between blood group polymorphisms, milk, and type traits in dairy cattle; to apply modern theoretical designs and statistical models to the largest data set ever available for this type of study in an attempt to assess the utility of blood group polymorphisms as selection aids in current Holstein breeding schemes; and to integrate the results obtained with the findings of earlier studies in an attempt to formulate general conclusions relative to the approach of resolving quantitative variation into individual locus effects. These conclusions could be of value to the many projects in preparation or in progress in the area of QTL mapping.

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¹To whom correspondence should be addressed.

MATERIALS AND METHODS

Quantitative Data

The quantitative data provided by the HA consisted of three subsets: a bull file containing PTA for milk production and conformation traits of all bulls registered and blood typed since the 1940s; a cow file on milk production containing information for first lactations of cows that were registered with the HA and blood typed between the late 1950s and the late 1980s and on untyped contemporaries of these cows; and a file on cow type and conformation containing visual appraisal scores for type and conformation traits of cows registered and blood typed between

1979 and 1989 and of nontyped contemporaries of these cows.

The bull file contained 57, 554, 1875, 10,145, and 9983 bulls born in the 1940s, 1950s, 1960s, 1970s, and 1980s, respectively. The traits for which PTA were available, their respective PTA means and standard deviations, the number of bulls with records, and other relevant information are in Table 1.

The data in the cow file on milk production were 305-d lactation, twice daily milking, mature equivalent (ME) records for first lactations longer than 200 d that were derived following DHIA guidelines. Means, standard deviations, and total number of records for the variables in this file are presented in Table 2. Of the cows in Table 2, only 98,685 had blood group genotypes. Means for these blood-typed (registered) cows were superior to those for both grade and registered cows in Table 2, in agreement with Short and Lawlor (39). The number of blood-typed cows that were born in the 1960s, 1970s, and 1980s were 1667, 20,422, and 76,596, respectively.

Of the 98,685 blood-typed cows, only 53,877 had maternal grandsire information, which was utilized in the family design (44) and direct effects analyses. In the granddaughter design (51), sons of heterozygous sires were scored for markers, and the quantitative values of granddaughters were contrasted. Because blood group information on the cows was not required, the data utilized in the granddaughter design analyses were for 364,228 cows with maternal grandsire information. The data in Table 2 are representative of both of these subsets, the data structure of which is characterized in Table 3.

Birth, pedigree, linear scores for 14 type traits, final type score (40, 47), and herd, year, and season of visual classification for a total of 447,800 cows born between 1979 and 1989 were in the cow file on type and conformation. Means, standard deviations, and numbers of records for the conformation traits are in Table 4. These cows were utilized in the granddaughter design analyses, but the family design and direct effects analyses utilized only 61,420 cows with blood

TABLE 1. Bull file descriptive parameters.¹

| Variable ² | n | \bar{X} | SD |
|--|--------|-----------|--------|
| Production traits | | | |
| Milk, kg | 22,460 | -111.0 | 458.2 |
| Milk fat, kg | 22,460 | -3.1 | 14.8 |
| Reliability milk and fat, %³ | | | |
| Daughters per bull, ⁴ no. | 22,460 | 363.2 | 1806.4 |
| Herds per bull, ⁴ no. | 22,460 | 140.3 | 530.6 |
| Protein, kg | 17,749 | 0.20 | 10.8 |
| Protein, \$ | 17,749 | 2.4 | 102.1 |
| Reliability protein, ³ % | 17,749 | 71.5 | 14.6 |
| Conformation traits⁵ | | | |
| Body depth | 11,175 | 0.47 | 1.46 |
| Dairy form | 11,175 | 0.98 | 1.44 |
| Foot angle | 11,175 | 0.22 | 0.81 |
| Fore udder attachment | 11,175 | 0.11 | 1.17 |
| Front teat placement | 11,175 | 0.21 | 1.19 |
| Rear leg set | 11,175 | -0.11 | 0.95 |
| Rear udder height | 11,175 | 0.46 | 1.15 |
| Rear udder width | 11,175 | 0.47 | 1.06 |
| Rump angle | 11,175 | 0.03 | 1.11 |
| Stature | 11,175 | 0.35 | 1.82 |
| Strength | 11,175 | 0.21 | 1.31 |
| Thurl width | 11,175 | 0.21 | 1.25 |
| Udder cleft | 11,175 | 0.38 | 0.94 |
| Udder depth | 11,175 | 0.05 | 1.05 |
| Reliability linear traits, ⁶ % | 11,175 | 66.6 | 14.1 |
| Overall conformation score | 14,899 | 0.34 | 0.86 |
| Reliability conformation, ⁷ % | 14,899 | 65.3 | 15.5 |
| Daughters per bull ⁸ | 14,899 | 155.6 | 955.0 |
| Herds per bull ⁸ | 14,899 | 72.1 | 302.3 |

¹The file comprised 796 paternal half-sib families with an average of 27.2 male sibs per family and included 2745 maternal grandsires.

²Trait values are PTA.

³Genetic evaluation based on a two-trait model.

⁴In the evaluation of genetic merit for milk and fat yields.

⁵Units of type traits are points scored on a linear scale.

⁶Genetic evaluation based on a 14-trait model.

⁷Genetic evaluation based on a single-trait model.

⁸In the evaluation of genetic merit for overall type score.

TABLE 2. Descriptive parameters for milk production from the cow data file.

| First lactation ¹ | n | \bar{X} | SD |
|------------------------------|-----------|-----------|--------|
| Milk, kg | 1,924,171 | 8602.2 | 1686.3 |
| Milk fat, kg | 1,924,171 | 309.7 | 61.1 |
| Milk protein, kg | 978,732 | 282.3 | 49.7 |
| Milk fat, % | 1,924,171 | 3.6 | 0.40 |
| Milk protein, % | 978,732 | 3.1 | 0.20 |
| Protein:fat | 978,732 | 0.88 | 0.09 |

¹For a 305-d lactation, twice daily milking, mature equivalent milk production.

group genotypes. Descriptive parameters for these data were similar to those in Table 4. Information pertaining to the structure of these data subsets is presented in Table 3.

Blood Group Data

Two files were provided by the HA, blood group genotypes of 22,614 bulls registered and blood-typed since the 1940s, and blood group genotypes of 168,166 cows registered and blood-typed since the late 1950s. Both files contained genotype information for 10 blood groups (A, B, C, F, L, M, S, R', T', and Z) and electrophoretically detected polymorphisms of the protein transferrin. Dominance relationships among alleles resulted in a number of incomplete genotypes; for some animals, only one allele or haplotype had been ascertained. In these cases, homozygous dominant individuals could not be distinguished from heterozygotes for the recessive null allele. Pedigree information was used to resolve these ambiguous genotypes; however, a number of incomplete genotypes in the male and female samples remained for

TABLE 3. Information pertaining to data subsets utilized in the different designs.

| | Herd-year-seasons ¹ | Families ² | Mean half-sibs per family |
|-----------------------------------|---------------------------------|-----------------------|---------------------------|
| | — Cow milk production (no.) — | | |
| Blood-typed cows ³ | 33,171 | 2683 | 34.8 |
| Daughter design ⁴ | 28,923 | 945 | 55.3 |
| Granddaughter design ⁵ | 57,542 | 11,900 | 29.9 |
| | Cow type and conformation (no.) | | |
| Blood-typed cows ⁶ | 22,485 | 1032 | 57.8 |
| Granddaughter design ⁷ | 22,485 | 14,127 | 31.1 |

¹Of first calving in the case of the cow milk production file and of visual classification in the case of the cow type and conformation file.

²Families of paternal half-sibs.

³There were 98,685 blood-typed cows.

⁴There were 53,877 blood-typed cows with maternal grandsire information that were also the subset utilized in the direct effects analyses. There was a total of 3718 maternal grandsires in this data subset.

⁵There were 364,228 cows with maternal grandsire information. No blood group genotypes of cows are required in this design. There was a total of 24,208 maternal grandsires in this data subset.

⁶There were 61,420 blood-typed cows that were utilized in the daughter design and direct effects analyses. There was a total of 4023 maternal grandsires in this data subset.

⁷All 447,800 cows in this file possessed maternal grandsire information and were utilized in the granddaughter design analyses. There was a total of 27,964 maternal grandsires in this file.

TABLE 4. Descriptive parameters for type and conformation traits.

| Variable ^{1,2} | \bar{X} | SD |
|--------------------------|-----------|-----|
| Body depth | 29.6 | 7.4 |
| Dairy form | 28.9 | 7.3 |
| Foot angle | 23.6 | 6.4 |
| Fore udder attachment | 24.0 | 7.2 |
| Front teat placement | 23.5 | 6.3 |
| Rear leg set | 27.0 | 6.6 |
| Rear udder height | 25.3 | 7.2 |
| Rear udder width | 25.2 | 7.2 |
| Rump angle | 24.5 | 5.3 |
| Stature | 31.4 | 8.4 |
| Strength | 27.6 | 7.1 |
| Thurl width | 28.3 | 7.4 |
| Udder cleft | 26.8 | 5.9 |
| Udder depth | 22.3 | 4.6 |
| Final conformation score | 81.4 | 4.4 |

¹447,800 observations for all traits.

²Units of type traits are points on a linear scale: from 50 to 100 for final score and from 1 to 50 for all other traits.

the different blood group polymorphisms that are presented in Table 5. Rocha (33) characterized these data with respect to allele frequencies, polymorphism information content, Hardy-Weinberg equilibrium, genotypic disequilibrium among pairs of loci (50), and allele frequency changes over time. Of these results, only those relevant to detected QTL associations are reported.

A Strategy of Consistency for QTL Detection

The available data were from one breed, spanned a period of over 40 yr during which selection was practiced, and included many large half-sib sire families of both males (with PTA data) and females (with phenotypic records). Some of these half-sib families were extraordinarily large (>1000 sibs) as were many granddaughter-paternal grandsire families (with phenotypic records). In consideration of these elements and the numerous statistically significant results in the literature that are often inconsistent across experiments, the following strategy was designed to detect biologically meaningful marker-QTL associations.

1. Perform nested ANOVA family design analyses for both male (PTA data) and female (phenotypic data) half-sib families out of heterozygous sires. Families with fewer than 5 sibs in the least numerous of the two allelic classes were excluded.

TABLE 5. Numbers of incomplete genotypes in males and females.¹

| Locus | Incomplete genotypes | | Missing genotypes | |
|-------|----------------------|-------------------|-------------------|---------|
| | Males | Females | Males | Females |
| | (no.) | | | |
| A | 7819 | 56,451 | 18 | 90 |
| B | 2745 | 16,446 | 72 | 212 |
| C | 7417 | 40,816 | 54 | 148 |
| F | 31 ² | 107 ² | 3 | 62 |
| L | 4578 | 28,253 | 12 | 73 |
| M | 1754 | 1716 | 1092 | 336 |
| R' | 535 ² | 1912 ² | 4722 | 13,489 |
| S | 14,162 | 103,822 | 38 | 154 |
| T' | 1616 | 20,315 | 19,791 | 80,956 |
| Z | 2998 | 25,216 | 17 | 94 |
| TF | 0 | 1 | 22,437 | 168,166 |

¹Data were from 22,614 bulls and 168,166 cows.

²Codominant systems; some genotypes were incomplete because they were determined only from pedigree information.

2. Apply granddaughter designs to the families of cows out of heterozygous paternal grandsires. These designs involved a hierarchical statistical analysis with allele classification nested within heterozygous grandsire and sire nested within allele classification within heterozygous grand-sire.
3. Perform analyses aimed at the detection of pleiotropic (direct) effects of blood group antigens. Marker-genotype contrasts were tested for

TABLE 6. Number of families and sample sizes for the family designs.

| Blood group ¹ | Families | | | Total sample size | | |
|--------------------------|--------------------|----------------------|-----------------|--------------------|----------------------|-----------------|
| | Males ² | Females ² | GD ³ | Males ⁴ | Females ⁴ | GD ⁵ |
| | (no.) | | | | | |
| A | 56 | 79 | 35 | 4932 | 16,978 | 127,997 |
| B | 127 | 167 | 75 | 12,527 | 42,110 | 223,029 |
| C | 102 | 151 | 53 | 9952 | 35,470 | 263,449 |
| F | 22 | 31 | 15 | 2151 | 7339 | 27,816 |
| L ⁶ | 43 | 69 | 26 | 4453 | 17,593 | 119,654 |
| L ⁷ | 50 | 70 | 22 | 5088 | 16,722 | 34,536 |
| M ⁶ | 2 | 2 | 1 | 468 | 126 | 3119 |
| M ⁷ | 2 | 1 | 1 | 457 | 48 | 2826 |
| R' ⁶ | 3 | 4 | 3 | 701 | 1974 | 14,796 |
| R' ⁷ | 3 | 3 | 3 | 519 | 1010 | 13,713 |
| S | 69 | 88 | 36 | 3236 | 10,988 | 153,100 |
| T' ⁶ | 7 | 19 | 4 | 149 | 4644 | 3704 |
| T' ⁷ | 4 | 29 | 2 | 211 | 6936 | 209 |
| TF | 2 | ... | 2 | 23 | ... | 8037 |
| Z | 39 | ... | 24 | 2031 | ... | 17,199 |
| Z ⁶ | ... | 62 | ... | ... | 11,253 | ... |
| Z ⁷ | ... | 63 | ... | ... | 12,083 | ... |

¹Single-marker family analyses.

²Half-sib families out of heterozygous sires. Families with fewer than 5 progeny in the smallest of the two allele classes were excluded.

³Granddaughter families with heterozygous paternal grandsires. Families with fewer than 5 second-generation sires in the smallest of the two allele classes were excluded.

⁴Some heterozygous progeny were excluded.

⁵Some heterozygous second-generation sires and their daughters were excluded. Sire groups with fewer than 50 daughters were excluded.

⁶Analyses based on heterozygous progeny.

⁷Analyses based on progeny with incompletely ascertained genotypes.

the population rather than within families. Genotypes with fewer than 15 animals were excluded from these analyses. Single- and multiple-marker models were fitted, and two-way epistatic interactions between all pairs of markers (excluding the complex B and C systems) were evaluated.

4. Perform multivariate analyses [MANOVA procedure of SAS; (16)] to address the problems from correlations among the dependent variables and from the inflation in experimental Type I error rates when multiple statistical tests were performed (52).

The analyses described were conducted across all families and based on animals with complete genotype information, and progeny with the same genotype as the heterozygous sire (or grandsire) were excluded in the family (and granddaughter) design analyses, except for a few cases that are discussed herein. Tests of normality were performed for all variables, and residual and outlier analyses were performed for all models fitted. Although all significant results are reported, only the associations that were consistently detected across analyses were interpreted as being biologically valid and were evaluated for the magnitude of quantitative effects and the proportions of phenotypic and genetic variances that they explain.

Criteria to summarize the 45 yr of research in this area were as follows: the results of direct effects analyses, which did not include mixed model approaches and procedures to control the experimental Type I error, were only considered relevant if significant at $P < 0.001$; results of family design analyses, which did not include protection against inflated Type I error rates, were only considered relevant if significant at $P < 0.01$; otherwise, $P < 0.05$ was considered the threshold for relevance; also, if a consistent pattern of association between a blood group and a quantitative trait was evident across studies, $P < 0.05$ was again considered relevant. Effects of overall blood group heterozygosity on quantitative traits were examined.

Statistical Models

PTA data for bulls. The model utilized in the direct effects analyses was

$$Y_{ijkl} = \mu + A_i + B_j + C_k + e_{ijkl} \quad [1]$$

where

Y_{ijkl} = PTA value for bull l of blood group genotype i and by sire j and maternal grandsire k ,

μ = constant common to all observations,

A_i = fixed direct genotype effect associated with the genotype i of a blood group—for PTA, heterozygotes should have values intermediate to homozygotes,

B_j = random effect of sire j ,

C_k = random effect of maternal grandsire k , and

e_{ijkl} = random error associated with Y_{ijkl} assumed identically and independently distributed $\sim N(0, \sigma_e^2)$.

These models were fitted by SAS GLM (16). Maternal grandsires were absorbed, and weighted least squares with PTA reliabilities as weights were utilized. For the multivariate analyses, Wilk's criterion (16) was utilized to test the multivariate null hypothesis that none of the blood group genotype means differed for any trait. When the multivariate null hypothesis was rejected, univariate F tests were used to determine which genotypes and dependent variable or variables were responsible for the differences (33). For the multivariate analyses, SAS (16, 37) requires that there be no missing values for any dependent variable. Together with the weighted least squares approach, which required the utilization of different reliabilities for different sets of PTA values (see Table 1), this restriction led to fitting four different models: Model 1-M, where M indicates male, included PTA for milk and PTA for milk fat as the dependent variables; Model 2-M included PTA for milk protein and PTA dollars for milk protein; 3-M was a univariate model for PTA overall type score; and Model 4-M included PTA for all 14 linear type measurements in Table 1. Percent-ages of milk fat and protein have historically been evaluated for marker-QTL associations, and Gonyon et al. (19) and Haenlein et al. (20) also studied the ratio of milk protein to fat. To evaluate these traits for which PTA were not available, models for PTA for milk fat (Model 5-M) and protein traits (Model 6-M) were fitted that included a covariate for PTA for milk yield and, finally, Model 7-M was fitted for PTA milk protein traits including a covariate for PTA milk fat yield. The error term for all tests of hypotheses was the residual error.

In the family designs, the term A_i in [1] was replaced by $A_{i(j)}$, a fixed effect associated with the inheritance of allele i (for a given blood group) from the sire j by his male progeny. Numbers of male half-

sib sire families and total sample sizes included in these analyses are presented in Table 6. Heterozygous progeny with the same genotype as their sire were retained for the L, M, R', and T' blood group systems. In these dominant two-allele systems, only a few homozygous dominant individuals were ascertained, and the exclusion of offspring with heterozygous genotypes would have rendered the analysis infeasible. To overcome this problem, two types of family analyses were performed for each of these systems. One analysis was based on within-family contrasts between the heterozygous and homozygous recessive genotypes. The expected value of this contrast was less than when alternate homozygotes are utilized, and, therefore, the statistical power of the test was reduced. However, the detection of quantitative differences should be possible given the large available sample sizes. An alternative analysis was based on offspring with incompletely ascertained genotypes (Tables 5 and 6) by establishing within-family contrasts between the heterozygous and homozygous dominant (pooled) and homozygous recessive offspring (excluding from the analyses those offspring known to be heterozygous). The expected value of this contrast was greater than in the previous case if a QTL was linked to the blood group.

Phenotypic data for first lactation cows. The model utilized in the direct effects analyses was

$$Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + e_{ijklm} \quad [2]$$

where

Y_{ijklm} = milk production or type and conformation trait score for cow m in herd-year-season i and with blood group genotype j by sire k and with maternal grandsire l ,

μ = constant common to all observations,

A_i = fixed herd-year-season effect associated with the herd-year-season i . Year and season refer to the first calving for milk production, or to the classification of the cow for type and conformation traits,

B_j = fixed genotype effect associated with genotype j of a given blood group,

C_k = random effect for sire k ,

D_l = random effect for maternal grandsire l , and

e_{ijklm} = random error associated with Y_{ijklm} assumed identically and independently distributed $\sim N(0, \sigma_e^2)$.

Models were fitted by ordinary least squares using the GLM procedure of SAS (16, 37), and herd-year-

season effects were absorbed. The same multivariate strategy was followed as described for the bull models. Because of differences in the number of observations available for the different variables (Tables 2 and 4), different multivariate models were fitted: Model 1-F, where F indicates female, included milk yield, milk fat yield, and milk fat percentage as the dependent variables; Model 2-F included milk protein yield, milk protein percentage, and the ratio of milk protein to fat; and Model 3-F included all 15 type and conformation traits. In the family design analyses, the term B_j in [2] was replaced by a nested term $B_{j(k)}$, a fixed effect associated with the inheritance of allele j (for a given blood group) from the sire k by his female offspring. Numbers of female half-sib families and total sample sizes included in these analyses are presented in Table 6.

Granddaughter designs. This design was applied to granddaughter families that were based on heterozygous paternal grandsires for a particular blood group. The model utilized was

$$Y_{ijklmn} = \mu + A_i + B_j + C_k + D_{l(k)} + F_{m(l(k))} + e_{ijklmn} \quad [3]$$

where

Y_{ijklmn} = milk production or type and conformation trait score for cow n , out of sire m , of allelic classification l , within the paternal grandsire family k , and with maternal grandsire j , and in herd-year-season i ;

μ = constant common to all observations;

A_i = fixed herd-year-season effect associated with the herd-year-season i ;

B_j = random effect due to maternal grandsire j ;

C_k = random effect due to the paternal grandsire k ;

$D_{l(k)}$ = fixed allelic inheritance effect associated with the inheritance of allele l (for a given blood group) from the paternal grandsire k by his male offspring;

$F_{m(l(k))}$ = random effect due to the sire m nested within the allelic classification l (for a given blood group) nested within the paternal grandsire family k ; and

e_{ijklmn} = random error associated with Y_{ijklmn} assumed identically and independently distributed $\sim N(0, \sigma_e^2)$.

The procedures that have been described for the other female-based analyses were followed. Because

the blood group genotypes of granddaughters were not required in this design, all of the guidelines defined for the implementation of the male family designs also applied here. Sire-groups with fewer than 50 cows were excluded from the analyses. Numbers of paternal grandsire families and sample sizes included in the granddaughter designs for the different blood groups are presented in Table 6.

A major difference between the implementation of the granddaughter and family designs concerned the error terms that were utilized to test the hypotheses. Although not acknowledged by Weller et al. (51), the appropriate error term to test hypotheses regarding QTL effects (allele inheritance effects nested within paternal grandsire families) under a granddaughter design is the random effect of sire nested within allelic classification nested within paternal grandsire family. To test univariate hypotheses, the construction of artificial mean squares across families was required (33) because the coefficients of the variance components varied among the different expected mean squares.

Model Adequacy and Normality Checks

The distribution of each of the dependent variables was evaluated using the SAS UNIVARIATE procedure (15). Normal probability (Q-Q) plots, histograms, skewness, and kurtosis values were examined to determine whether any of the distributions deviated from normality. For the assumptions of independence, normality, and homoscedasticity of model residuals, plots of predicted against residual values, and histograms of residual values were obtained for each of the models to assess the distribution shapes, the occurrence of nonrandom patterns of residuals, the presence of outliers, and the constancy of the residual variance across the range of the predicted values.

Additional Analyses for Consistently Detected Marker-QTL Effects

For the marker-QTL associations that consistently emerged from the different analytical approaches, the magnitude of the quantitative effect was estimated by different procedures. Least squares estimates of genotypic effects were obtained from the direct effects analyses, and averages of the absolute values of the least squares estimates of significant within-family contrasts were computed from the family design analyses. The proportions of phenotypic and additive genetic variance accounted for by the markers were computed by Henderson's method 3 from the expected mean squares produced using the SAS RANDOM

statement (16). Consistently detected effects from previous research were also evaluated in these data.

Assessment of Overall Blood Group Heterozygosity Effects

Quantitative effects of overall blood group heterozygosity were assessed using female phenotypic data and male PTA data (to detect additive by additive epistatic effects). The models that were fitted and the statistical strategy used were as for the direct effects analyses, except that the marker effect was replaced with linear and quadratic regressions on the number of blood groups for which an animal was heterozygous (0 through 8; blood groups S, T', and TF were excluded to avoid a reduction in the sample size; Table 5).

RESULTS

Previous Research

Of the 301 statistically significant associations previously reported, 3 were sufficiently consistent across studies to suggest their biological validity; the associations of the B blood group and milk fat percentage (1, 10, 27, 29, 30, 31, 33, 48, 49); the L blood group and milk composition traits (fat, protein, casein and solids-not-fat percentages), in most cases with concomitant reciprocal effects on milk yield (1, 19, 20, 27, 30, 31, 33); and the M blood group and milk yield (1, 19, 20, 21, 27, 33, 48, 49), possibly as a consequence of linkage of this locus to the bovine lymphocyte antigen (**BoLA**) system, which affects mastitis resistance (25, 26, 42). Some of the effects of the M system on milk yield may be mediated through effects on DMI (3) and may also affect milk composition traits (percentages of fat, protein, and solids not fat). Many studies (1, 3, 4, 5, 6, 10, 18, 19, 20, 23, 33, 49) have discussed an effect of the transferrin locus on milk yield. However, our criteria for consistency have led us to place less credibility on this putative association than on the other 3 (33). Two-way epistatic interactions were evaluated and detected in two recent studies (19, 20). Those interactions that appear to be biologically consistent involve the L and M blood groups. Heterozygosity effects of blood group on milk production traits have not been found to be important (19, 20, 29, 33).

Evolution of Allele Frequencies Across Decades

Linear trends in allele frequencies across decades were detected for some of the alleles of the A, B, C, L,

TABLE 7. Time trends in blood group allele frequencies.

| Allele | 1950s ¹ | 1960s | 1970s | 1980s | 1980 -1984 | 1985 -1989 |
|------------------------------------|--------------------|-------|-------|-------|---------------|---------------|
| Blood group C males ² | | | | | | |
| Null | 0.10 | 0.20 | 0.04 | 0.01 | ... | ... |
| C ₁ E | 0.16 | 0.14 | 0.19 | 0.22 | ... | ... |
| WX ₂ C'' | 0.04 | 0.05 | 0.09 | 0.12 | ... | ... |
| Blood group C females ² | | | | | | |
| Null | ... | 0.07 | 0.03 | 0.01 | 0.01 | 0.01 |
| C ₁ E | ... | 0.14 | 0.17 | 0.20 | 0.20 | 0.21 |
| WX ₂ C'' | ... | 0.07 | 0.09 | 0.11 | 0.12 | 0.11 |
| Blood group L males ³ | | | | | | |
| Null | 0.78 | 0.77 | 0.79 | 0.73 | ... | ... |
| L | 0.22 | 0.23 | 0.21 | 0.27 | ... | ... |
| Blood group L females ³ | | | | | | |
| Null | ... | 0.80 | 0.80 | 0.76 | 0.79 | 0.75 |
| L | ... | 0.20 | 0.20 | 0.24 | 0.21 | 0.25 |
| Blood group M males ³ | | | | | | |
| Null | 0.88 | 0.88 | 0.98 | 0.99 | ... | ... |
| M ₂ M' | 0.12 | 0.12 | 0.02 | 0.01 | ... | ... |
| Blood group M females ³ | | | | | | |
| Null | ... | 0.96 | 0.98 | 0.99 | 0.99 | 0.99 |
| M ₂ M' | ... | 0.04 | 0.02 | 0.01 | 0.01 | 0.01 |

¹This group includes the few bulls born in the 1940s.

²Biased estimates of frequencies obtained by allele counting.

³Biased estimates of frequencies obtained by the square root method.

M, and Z blood group systems (33), but only those for the C, L, and M systems could be related to quantitative effects associated with these blood groups. The changes in allele frequencies that were observed for these three groups are presented in Table 7.

Direct Effects Analyses

Fifty-one (59%) of a total of 86 multivariate hypothesis tests conducted for males (PTA data) and females (phenotypic data) and for milk production and for type and conformation traits were significant

($P < 0.05$), and 33 (65%) of these tests were highly significant ($P < 0.001$). Of the univariate tests, 155 (31%) of 498 were significant ($P < 0.05$), and 70 (45%) of these tests were highly significant ($P < 0.001$). This preponderance of statistically significant results supports the conclusion of Kennedy et al. (24) that least squares methods (that ignore genetic covariances among relatives) that were not based on special family designs lead to the detection of spurious QTL effects. The inclusion of effects for sire and maternal grandsire in these models appears not to have been sufficient to circumvent this problem, and,

TABLE 8. Two-locus blood group epistatic interactions.

| Interaction ¹ | Trait | Model | R ² | Multivariate P | Univariate P |
|--------------------------|------------------------------|-------|----------------|-------------------|-----------------|
| A × L | PTA for milk yield | 1-M | 0.76 | 0.03 | 0.005 |
| | PTA for milk fat yield | 1-M | 0.72 | 0.03 | 0.05 |
| | PTA for milk protein yield | 2-M | 0.71 | 0.01 | 0.003 |
| | PTA for milk protein dollars | 2-M | 0.73 | 0.01 | 0.003 |
| | PTA for dairy form | 4-M | 0.62 | 0.05 | 0.01 |
| A × F | PTA for udder cleft | 4-M | 0.62 | 0.0001 | 0.007 |
| A × S | PTA for foot angle | 4-M | 0.70 | 0.02 | 0.003 |
| L × M | Dairy form phenotype | 3-F | 0.84 | 0.005 | 0.01 |

¹Blood groups involved in the two-way epistatic effect.

therefore, the results of these analyses are considered only when they support the results of the other analytical approaches. Average coefficients of determination (R^2) and ranges, in parentheses, for the models fitted in these analyses were 0.84 (0.58 to 0.9997), 0.63 (0.42 to 0.9996), 0.87 (0.71 to 0.93), and 0.82 (0.79 to 0.89) for PTA models for milk production, PTA models for type and conformation, phenotypic models for milk production, and phenotypic models for type and conformation, respectively. Few of the two-way epistatic interactions were significant ($P < 0.05$), but these are presented in Table 8.

Male Family Designs

Associations ($P < 0.05$) of effects of the male family designs are presented in Table 9. Mean values for coefficients of determination (R^2) and ranges were 0.75 (0.31 to 0.999) and 0.55 (0.25 to 0.998) for models for milk production and for type and conformation traits, respectively.

Female Family Designs

Results ($P < 0.05$) of the effects of the female family designs are presented in Table 10. Mean

TABLE 9. Significant associations detected in the male family designs.

| Locus | PTA Trait | Model | R^2 | Multivariate P | Univariate P |
|-------|-------------------------------------|-------|-------|------------------|----------------|
| A | Milk yield | 1-M | 0.71 | 0.02 | 0.01 |
| B | Milk fat yield | 1-M | 0.70 | 0.03 | 0.03 |
| | Milk fat yield ¹ | 5-M | 0.82 | ... | 0.04 |
| | Rear udder height | 4-M | 0.55 | 0.0001 | 0.02 |
| | Rear udder width | 4-M | 0.55 | 0.0001 | 0.03 |
| | Udder depth | 4-M | 0.54 | 0.0001 | 0.03 |
| | Rear leg set | 4-M | 0.52 | 0.0001 | 0.05 |
| | Foot angle | 4-M | 0.65 | 0.0001 | 0.05 |
| | Dairy form | 4-M | 0.60 | 0.0001 | 0.05 |
| C | Milk yield | 1-M | 0.67 | 0.04 | 0.01 |
| | Milk fat yield | 1-M | 0.64 | 0.04 | 0.05 |
| | Milk protein yield | 2-M | 0.63 | 0.0001 | 0.004 |
| | Milk protein dollars | 2-M | 0.65 | 0.0001 | 0.002 |
| | Milk protein yield ¹ | 6-M | 0.92 | 0.007 | 0.005 |
| | Udder cleft | 4-M | 0.53 | 0.0001 | 0.0001 |
| | Front teat placement | 4-M | 0.51 | 0.0001 | 0.0001 |
| | Foot angle | 4-M | 0.58 | 0.0001 | 0.0004 |
| | Rump angle | 4-M | 0.39 | 0.0001 | 0.003 |
| | Thurl width | 4-M | 0.49 | 0.0001 | 0.0001 |
| | Dairy form | 4-M | 0.56 | 0.0001 | 0.03 |
| | Body depth | 4-M | 0.46 | 0.0001 | 0.0006 |
| | Strength | 4-M | 0.44 | 0.0001 | 0.002 |
| | Stature | 4-M | 0.47 | 0.0001 | 0.0005 |
| F | Front teat placement | 4-M | 0.49 | 0.005 | 0.02 |
| | Thurl width | 4-M | 0.56 | 0.005 | 0.05 |
| | Stature | 4-M | 0.55 | 0.005 | 0.01 |
| L | Milk protein yield ¹ | 6-M | 0.92 | 0.04 | 0.04 |
| | Milk fat yield ² | 1-M | 0.72 | 0.001 | 0.002 |
| | Milk fat yield ^{1,2} | 5-M | 0.81 | ... | 0.0003 |
| | Milk protein yield ^{1,2} | 6-M | 0.93 | 0.0001 | 0.0001 |
| | Milk protein dollars ^{1,2} | 6-M | 0.95 | 0.0001 | 0.0001 |
| S | Milk yield | 1-M | 0.73 | 0.0001 | 0.0001 |
| | Milk fat yield | 1-M | 0.68 | 0.0001 | 0.0001 |
| | Milk fat yield ¹ | 5-M | 0.80 | ... | 0.03 |
| | Milk protein yield | 2-M | 0.69 | 0.0001 | 0.007 |
| | Milk protein dollars | 2-M | 0.71 | 0.0001 | 0.0001 |
| | Foot angle | 4-M | 0.66 | 0.0001 | 0.02 |
| | Dairy form | 4-M | 0.56 | 0.0001 | 0.02 |
| | Strength | 4-M | 0.48 | 0.0001 | 0.02 |
| Z | Front teat placement | 4-M | 0.64 | 0.006 | 0.03 |
| | Rear leg set | 4-M | 0.65 | 0.006 | 0.04 |
| | Foot angle | 4-M | 0.70 | 0.006 | 0.0001 |

¹Model including PTA for milk yield as a covariate.

²Model based on offspring with incompletely ascertained genotypes.

TABLE 10. Significant associations detected in the female family designs.

| Locus | Trait | Model | R ² | Multivariate <i>P</i> | Univariate <i>P</i> |
|-----------------|-----------------------|-------|----------------|--------------------------|------------------------|
| B | Udder depth | 3-F | 0.59 | 0.0003 | 0.04 |
| | Rump angle | 3-F | 0.54 | 0.0003 | 0.0006 |
| | Dairy form | 3-F | 0.59 | 0.0003 | 0.03 |
| | Body depth | 3-F | 0.61 | 0.0003 | 0.02 |
| C | Fore udder attachment | 3-F | 0.59 | 0.0001 | 0.0001 |
| | Rump angle | 3-F | 0.57 | 0.0001 | 0.004 |
| | Thurl width | 3-F | 0.62 | 0.0001 | 0.0002 |
| | Dairy form | 3-F | 0.62 | 0.0001 | 0.006 |
| | Body depth | 3-F | 0.63 | 0.0001 | 0.0001 |
| | Strength | 3-F | 0.62 | 0.0001 | 0.0001 |
| | Stature | 3-F | 0.64 | 0.0001 | 0.0001 |
| L | Milk yield | 1-F | 0.84 | 0.002 | 0.01 |
| | Milk protein percent | 2-F | 0.85 | 0.0001 | 0.0001 |
| R' ¹ | Thurl width | 3-F | 0.90 | 0.03 | 0.05 |
| | Body depth | 3-F | 0.91 | 0.03 | 0.04 |
| | Strength | 3-F | 0.89 | 0.03 | 0.006 |
| | Stature | 3-F | 0.90 | 0.03 | 0.04 |
| S | Milk fat yield | 1-F | 0.91 | 0.0001 | 0.008 |
| | Milk fat percentage | 1-F | 0.88 | 0.0001 | 0.005 |

¹Models based on offspring with incompletely ascertained genotypes.

values for coefficients of determination and their ranges were 0.87 (0.70 to 0.998) and 0.76 (0.54 to 0.998) for models for milk production and for type and conformation traits, respectively.

Granddaughter Design

Associations ($P < 0.05$) of the effects of the granddaughter designs are presented in Table 11. Mean values for coefficients of determination and their ranges were 0.93 (0.79 to 0.99) and 0.86 (0.65 to 0.99) for models for milk production and for type and conformation traits, respectively.

Among the large number of significant results reported in Tables 9 to 11, only those associating the C blood group with rump angle, the L blood group with milk composition traits (Tables 7 and 8; literature review), and the S blood group with milk fat yield were completely consistent across the different analyses.

Model Adequacy and Normalcy Checks

All traits were normally distributed except for overall type and conformation score (both PTA and

TABLE 11. Significant associations from the granddaughter design analyses.

| Locus | Trait | Model | R ² | Univariate <i>P</i> ¹ |
|-------|-------------------------|-------|----------------|----------------------------------|
| B | Milk fat yield | 1-F | 0.93 | 0.05 > <i>P</i> > 0.01 |
| | Rump angle | 3-F | 0.80 | 0.01 > <i>P</i> |
| C | Milk protein percentage | 2-F | 0.89 | 0.05 > <i>P</i> > 0.01 |
| | Rump angle | 3-F | 0.79 | 0.05 > <i>P</i> > 0.01 |
| F | Body depth | 3-F | 0.92 | 0.05 > <i>P</i> > 0.01 |
| L | Milk fat percentage | 1-F | 0.92 | 0.01 > <i>P</i> |
| M | Rear udder height | 3-F | 0.84 | 0.05 > <i>P</i> > 0.01 |
| | Foot angle | 3-F | 0.86 | 0.01 > <i>P</i> |
| R' | Milk yield | 1-F | 0.85 | 0.05 > <i>P</i> > 0.01 |
| | Milk fat yield | 1-F | 0.86 | 0.05 > <i>P</i> > 0.01 |
| | Foot angle | 3-F | 0.66 | 0.05 > <i>P</i> > 0.01 |
| S | Milk fat yield | 1-F | 0.94 | 0.05 > <i>P</i> > 0.01 |
| | Front teat placement | 3-F | 0.83 | 0.05 > <i>P</i> > 0.01 |

¹The *F*-test denominator was an artificial mean square. No multivariate tests were conducted.

TABLE 12. Magnitude of consistent L blood group effects.

| Trait | Mean family effect ¹ | Standardized effect ² | Range of family effects |
|--|---------------------------------|----------------------------------|-------------------------|
| — Male family designs (PTA traits) — | | | |
| Fat yield, ³ kg | 4.5 | ... (0.30) | 2.6–6.0 |
| Protein yield, ³ kg | 2.6 | ... (0.24) | 0.6–8.4 |
| — Female family designs (phenotypes) — | | | |
| Milk yield, kg | 1122.0 | 0.67 (1.2) | 301.1–1816.7 |
| Protein percentage | 0.19 | 0.95 (...) | 0.04–0.56 |
| — Granddaughter designs (phenotypes) — | | | |
| Fat percentage | 0.61 | 1.5 (...) | 0.54–0.74 |

¹Absolute value of the difference between alternate progeny groups within a family averaged over families with significant ($P < 0.10$) contrasts.

²Proportion of the phenotypic (additive genetic) standard deviation attributed to the average family effect. For PTA traits only the latter is reported. No genetic standard deviations were available for percentage traits.

³The PTA for milk yield was a covariate in the model.

phenotypes) and the ratio of protein to fat; deviations from normality were detected in the residual plots obtained from the models fitted for these variables. These traits were not considered further in the study.

Evaluation of Consistently Detected Marker-QTL Associations

Effects of the L blood group on milk production and composition traits. Estimates of the magnitude of significant effects from the family designs are in Table 12, and evidence of linkage disequilibrium and the percentage of variation explained are in Table 13. These results indicate that the effects were due to a linked QTL, but that some linkage disequilibrium was present in the population; the null allele predominantly was associated with negative effects on production traits and positive effects on percentage traits. Historical selection for increased milk production should have selected against the null allele, and Table 7 reveals that its frequency has decreased over time in both males and females (from 0.78 to 0.73 in males; 0.80 to 0.75 in females). Previous research suggests that the effect is due to a linked QTL rather than to a direct effect of the blood group (1, 19, 20, 27, 30, 31, 33). In this case, selection acts upon the blood group through linkage disequilibrium rather than directly upon the L system alleles, which would explain the modest change in allele frequencies over time. Also, results in Table 8 suggest that epistatic effects associated with the L system may introduce conflicting selection pressures. Results

presented by Rocha et al. (35) also suggest some conflict between production and type traits for this system. In general, the significant effects for the different traits in Tables 12 and 13 were localized to the same families. This strongly suggests that the L blood group effect is volumetric and that milk yield is affected to a greater extent than are fat and protein yields.

There was a considerable difference in magnitude between the within-family contrasts of different sign: for PTA for fat yield (adjusted for PTA for milk yield), the mean value for within-family contrast for the 7 families in Table 13 was 4.8 kg versus 2.6 kg for the single family with a contrast of different sign; for PTA for protein yield (adjusted for PTA for milk yield), the 11 families in Table 13 had a mean value for within-family contrast of 2.8 kg versus 1.4 kg for the single family with a contrast of different sign; and for milk yield in cows, the mean values for contrasts for the two sets of 3 families in Table 13 were 1673 and 571 kg. These values represent approximately twice the magnitude in contrasts of one sign versus contrasts of the opposite sign, which could simply be a reflection of chance sampling events, but the values could also support the presence of multiple QTL or multiple alleles at one QTL in the chromosomal segment marked by the L blood group (35).

There is also strong evidence for linkage disequilibrium in the population from the results of the direct effects analyses in Table 14. Estimates in Table 14 are considerably smaller than those in Table 12 because of the different marker-QTL phase relationships in different families, which explains why the changes in the L blood group allele frequencies in Table 7 have been modest. Although the proportions of phenotypic and additive genetic variances that are explained by an effect of this magnitude are low (Table 13), this result could be explained by either a low or high frequency of the desirable QTL allele. When only those families with significant contrasts (i.e., segregating for the QTL) were considered, these variance proportions increased considerably (Table 13).

Effects of the C blood group on rump angle. Magnitudes of the effects of the C blood group on rump angle and the proportions of variance explained by the system are in Tables 15 and 16. Results of the within-family contrasts indicate that the effect is due to a linked QTL because the direction of the effects of specific blood group alleles is family-dependent. A limited degree of linkage disequilibrium appears to be present. Rocha (33) fitted gene substitution models and found that phenogroups X_2C'' and WX_2C'' were

TABLE 13. Linkage disequilibrium and variance explained by L blood group effects.

| Design and trait | Families ¹ (no.) | Linkage disequilibrium ² | Variance explained | |
|------------------------------------|--------------------------------|-------------------------------------|--------------------|---------------------------------|
| | | | Phenotypic | Within sire ³ (%) |
| Male family designs (PTA traits) | | | | |
| Fat yield, ⁴ kg | 8 | 7 ⁵ -1 ⁶ | 0.009 ⁷ | ... |
| Protein yield, ⁴ kg | 12 | 11 ⁵ -1 ⁶ | 0.017 | ... |
| Female family designs (phenotypes) | | | | |
| Milk yield, kg | 6 | 3-3 | 1.1 | 1.2 (49.2) |
| Protein percentage | 13 | 8 ⁵ -5 ⁶ | 1.0 | 1.0 |
| Granddaughter designs (phenotypes) | | | | |
| Fat percentage | 3 | 0-3 ⁶ | 7.6 | 8.0 |

¹Number of segregating families with contrasts ($P < 0.10$).

²Distribution of families by contrast sign. Under linkage equilibrium, the number of families with positive and negative contrasts are approximately equal.

³Percentage of the within-sire phenotypic variance explained by the marker effect. Estimate obtained only including families with significant contrasts in parentheses.

⁴The PTA for milk yield was a covariate in the model.

⁵Number of families in which the transmission of the null allele was associated with an advantage.

⁶Number of families in which the transmission of the L allele was associated with an advantage.

⁷Fraction of the additive genetic variance (adjusted for PTA for milk yield) explained by the marker.

associated with a numerical disadvantage for rump angle [tendency for lower set hooks and higher set pins (47)] on a population basis. The substitution effects were -0.07 ± 0.025 PTA units per X_2C'' copy ($P = 0.003$), -2.9 ± 1.0 phenotype units per X_2C'' copy ($P = 0.005$), -0.15 ± 0.03 PTA units per WX_2C'' copy ($P = 0.0001$), and -5.9 ± 1.2 phenotype units per WX_2C'' copy ($P = 0.0001$). Percentages of variance accounted for by the C system under the direct effects models were 0.007% of the additive genetic variance (model based on PTA) and 0.6% of the total phenotypic variance (model based on phenotype).

Effects of the S blood group on milk fat yield.

Magnitudes of the effects of the S blood group on milk

fat yield and the proportions of variance explained by the system are in Tables 15 and 16. The data in Table 15 suggest a major gene (28) affecting milk fat yield linked to the S blood group. However, this system explains only a small proportion of the phenotypic and additive genetic variances (Table 16), possibly because of a low or a high frequency of the desirable QTL allele or alleles because these variance proportions increase dramatically when only segregating families are considered (Table 16). Trends for allele frequencies over time were inconsistent (data not reported).

The discrepancy between the effect on PTA, a modest 6.0 kg (Table 15), and the large effects that

TABLE 14. Direct effects associated with the L blood group.

| Trait | Effect ¹ | Standardized effect ² | Variance explained | |
|----------------------------------|---------------------|----------------------------------|--------------------|----------|
| | | | Phenotypic | Additive |
| | | | (%) | |
| PTA for protein, ³ kg | -0.37 (0.08) | ... (0.03) | ... | 0.001 |
| Milk yield, kg | 176.6 (35.7) | 0.11 (0.19) | 0.3 | ... |
| Protein yield, kg | 3.5 (1.1) | 0.07 (0.16) | 0.01 | ... |
| Fat percentage | -0.05 (0.01) | 0.13 (...) | 0.6 | ... |

¹Least squares estimates and standard errors in parentheses of the difference between L null heterozygotes and null homozygotes.

²Fraction of the phenotypic (additive genetic) standard deviation attributed to the effect. No genetic standard deviations were available for percentage traits.

³The PTA for milk yield was a covariate in the model.

TABLE 15. Magnitude of the effects of the C and S systems in different designs.

| Design | Mean family effect ¹ | Standardized effect ² | Range of family effects |
|--|---------------------------------|----------------------------------|-------------------------|
| Effects of the C system on rump angle ³ | | | |
| Male families (PTA) | 0.74 | ... (0.67) | 0.20–1.9 |
| Female families | 3.1 | 0.59 (1.4) | 0.80–9.2 |
| Granddaughter | 3.1 | 0.59 (1.4) | 1.50–5.6 |
| Effects of the S system on milk fat yield | | | |
| Male families (PTA) | 6.0 | ... (0.41) | 1.6–13.4 |
| Female families | 78.7 | 1.3 (2.7) | 9.1–176.6 |
| Granddaughter | 39.7 | 0.65 (1.3) | 17.1–70.3 |

¹Absolute value of the difference between alternative progeny groups within a family averaged over families with contrasts ($P < 0.10$).

²Proportion of the phenotypic (additive genetic) standard deviation attributed to the mean for family effect. For PTA traits only the additive genetic is reported.

were estimated from the other designs in Table 15 lacks adequate explanation. There are studies (10, 18, 30, 33) that present evidence in support of an effect of S blood group on fat yield, but the reported effects and significance levels do not support the findings of this study. However, when all evidence is considered, a large effect on milk fat yield in the chromosomal segment surrounding the S blood group appears to be beyond question. The results indicate an effect from a linked QTL, but there appears to be some linkage disequilibrium with the transmission of the null allele being associated primarily with decreased fat yields (3 families with positive contrasts vs. 10 with negative contrasts) and with the transmission of the H' allele being associated with increased fat yields (17 families with positive contrasts vs. 4 with negative contrasts).

Another aspect that deserves consideration is the comparison between the magnitude of the effects as detected from the granddaughter and the daughter designs (Table 15). For the effect of the C group on rump angle, the magnitude of the effect was the same in both designs (Table 15), which would tend to indicate an effect involving a large degree of dominance. For the effect of S group, the magnitude of the effect detected in the granddaughter design was about half that detected in the daughter design (female families; Table 15), which was close to expectation under an additive QTL model.

Effects of B blood group alleles on milk fat percentage. Previous research strongly suggests a direct positive effect of the BO₁Y₂D' phenogroup on milk fat percentage (1, 10, 27, 29, 30, 31, 33, 48, 49), which may be supported by the results in Tables 9 and 11. To evaluate this effect, males and females

were classified as either homozygous for the allele or heterozygous or homozygous for alternate alleles; this genotypic classification was not significant ($P > 0.05$) for PTA for fat yield, milk fat yield, and percentage phenotypes in the direct effects analyses. However, a related B phenogroup, G₂O₁Y₂(I'') (which shares the linear O₁Y₂ subarray present in the BO₁Y₂D' haplotype), yielded significant results. Genotypic effects in the direct effects analyses were detected only for PTA fat yield ($P = 0.004$); the G₂O₁Y₂(I'') allele was associated with increased fat yields. The least squares estimate of the difference between homozygotes and heterozygotes for the G₂O₁Y₂(I'') allele was 5.6 ± 3.1 kg ($P = 0.08$) of PTA fat, and the difference between

TABLE 16. Percentage of variance explained by the effects of the C and S systems.

| Design | Families ¹ (no.) | Variance explained | |
|---|--------------------------------|--------------------|--------------------------|
| | | Phenotypic | Within sire ² |
| Effects of the C system on rump angle | | | |
| Male families (PTA) | 12 | 0.02 ³ | ... |
| Female families | 21 | 0.54 | 0.57 (30.5) |
| Granddaughter | 15 | 1.3 | 1.3 |
| Effects of the S system on milk fat yield | | | |
| Male families (PTA) | 9 | 0.01 | ... |
| Female families | 9 | 1.1 | 1.2 (36.2) |
| Granddaughter | 4 | 3.3 | 3.5 |

¹Number of segregating families with contrasts ($P < 0.10$).

²Percentage of the within-sire phenotypic variance explained by the marker effect. Estimate obtained only including families with significant contrasts in parentheses.

³Proportion of the additive genetic variance explained by the marker effect.

heterozygotes and alternative homozygotes was 0.95 ± 0.35 kg ($P = 0.01$) of PTA for fat. The proportion of the additive genetic variance explained by this effect was 0.003%. This small effect agrees with estimates in the literature and confirms that some B system alleles have direct effects on milk fat traits (see also Tables 9 and 11). Because both the BO_1Y_2D' and $G_2O_1Y_2(I'')$ phenogroups share the O_1Y_2 subarray, the direct effects on milk fat may be associated with the O and Y loci rather than with particular phenogroups (29). No changes in allele frequency over time were detected for any of these phenogroups.

Direct effects of the M blood group on milk yield. Previous research (1, 19, 20, 21, 25, 27, 33, 48, 49) has solidly established that the M_2M' allele is associated with a reduction in milk yield and with an increase in mastitis prevalence. Both associations would result in strong selection against this allele, which is supported by the results in Table 7. No consistently significant effects associated with this locus emerged from the family-based analyses (Tables 8 to 11), which may have been a consequence of the limited degree of polymorphism at this locus. The frequency of the M_2M' allele was only 0.015 in males and 0.009 in females (33). Direct effects analyses were then performed based on the evidence in the literature for the effects of the M system on milk yield. Results for PTA traits were not significant. However, for homozygous females, null genotypes showed an advantage in ME milk yield of 352 ± 188 kg during a 305-d lactation with twice daily milking over heterozygous M_2M' -null genotypes ($P = 0.06$). The magnitude of this effect is very close to the 330-kg average computed from the literature (1, 19, 20, 21, 27, 33, 48, 49) and represents 0.21 of a phenotypic standard deviation (σ_p) and 0.38 of an additive genetic standard deviation (σ_a). Homozygous null genotypes also had a ME milk protein yield advantage of 11.6 ± 5.5 kg during a 305-d lactation with twice daily milking over M_2M' -null heterozygotes ($P = 0.04$), which represents 0.23 σ_p and 0.54 σ_a . Proportions of the total phenotypic variance that were explained by these effects were 2.5% for milk yield and 3.5% for milk protein yield.

Decade analyses. Data from each of the decades were analyzed separately to determine the support for the detected direct effects in the different time periods. The severe imbalance in sample size across decades (see Materials and Methods) led to inconclusive results in most cases. The substitution effect for the C system WX_2C'' phenogroup on rump angle remained fairly constant across decades (-0.13 PTA units per WX_2C'' copy in the 1970s vs. -0.15 PTA units per

WX_2C'' copy in the 1980s). However, the substitution effect for the C system X_2C'' phenogroup decreased from -0.15 PTA units per X_2C'' copy in the 1970s to -0.02 PTA units per X_2C'' copy in the 1980s, and the substitution effect for the B system $G_2O_1Y_2(I'')$ phenogroup decreased from 2.1 kg of PTA for fat per $G_2O_1Y_2(I'')$ copy in the 1970s to 0.48 kg of PTA for fat per $G_2O_1Y_2(I'')$ copy in the 1980s. These results could be explained by changes in QTL allele frequencies, by variation in linkage disequilibrium between generations, or by environmental or epistatic effects.

Effects of Heterozygosity of the Blood Groups

Two linear effects of overall heterozygosity of the blood group systems on milk yield ($P = 0.03$) and milk fat percentage ($P = 0.02$) were detected from cow phenotypes ($P = 0.0008$ in the multivariate test). The estimated regression coefficients were 40.2 ± 18.9 kg per heterozygous blood group for milk yield ($0.02 \sigma_p$ and $0.04 \sigma_a$) and $-0.02 \pm 0.007\%$ per heterozygous blood group for milk fat percentage ($0.05 \sigma_p$). Consequently, cows that were heterozygous for all eight blood groups would be expected to be superior by 321.6 kg to completely homozygous cows for milk yield ($0.19 \sigma_p$ and $0.35 \sigma_a$) and 0.16% inferior for milk fat percentage ($0.40 \sigma_p$). High-order epistatic interactions among many different chromosomal segments impacting the phenotypic expression of production traits is a possible biological interpretation for these results. A related interpretation, which perhaps is more plausible, is that blood group heterozygosity is negatively correlated with the degree of inbreeding, and, hence, these results reflect inbreeding depression.

DISCUSSION

Forty-Five Years of Research

Of 69 studies since 1951 [reviewed by Rocha (33)] reporting 3664 statistical tests of the associations between blood groups and milk and type traits, remarkably, only 3 of 301 significant associations emerge as being consistent across studies. To our knowledge, none of these associations has been utilized in practical breeding applications. This pattern of inconsistency of detected marker-QTL associations and the apparent lack of application of any of the consistently detected associations suggest a need for the reevaluation of the objectives of this area of

research by the academic community. The need to elucidate the genetic and environmental mechanisms responsible for the abundant number of inconsistencies in detected effects is of some urgency. Similarly, the impediments to the application of consistently detected effects in the livestock industries must be identified and resolved.

Recommendations for Holstein Breeding Schemes

To maximize the likelihood of useful applications for marker-assisted selection (MAS) in the short-term, this study adopted a strategy that required consistency of results (55) across different analytical designs. This requirement, we rationalized, would simultaneously emphasize the biological validity of the associations detected and identify associations that are robust to environmental and background genetic effects and that, therefore, would be most likely to be useful in the short-term. An important limitation of this strategy is that some potentially useful effects will not be detected. Of special concern would be QTL alleles of large effect but occurring at low frequencies in the population. Thus, the recommendations from this study may have overlooked important effects to be found in these data. However, prevention would have required that the analysis be performed on an individual family basis with further pursuit of every association detected in every family. This approach would likely have generated a considerable number of associations of lesser biological and production relevance, which would not have contributed to the successful implementation of MAS in the dairy industry.

The following results appear to be supported by strong evidence. The M_2M' allele of the M blood group is directly associated with a negative effect on milk and protein yields. The magnitude of the effects were estimated to be -352 kg for ME milk yield during a 305-d lactation with twice daily milking, and -11.6 kg for ME protein yield during a 305-d lactation with twice daily milking. These results are supported by the literature (1, 19, 20, 21, 27, 33, 48, 49), which suggests that the effects may be mediated by mastitis susceptibility. The M blood group is closely linked to the BoLA system (26), and there is evidence of strong linkage disequilibrium associating the M_2M' allele and the BoLA antigen w16, which is associated with an increased prevalence of mastitis (25, 26, 42). Further, the blood group antigen M' is actually a major histocompatibility complex class I-like molecule, possibly a remnant of BoLA-w16 (22). This effect is

remarkably consistent, both in the literature and in some of the analytical approaches implemented in this study [Table 7 and Rocha et al. (35)]. Unfortunately, the frequency of the M_2M' allele is very low in the US Holstein population (Table 7), and little genetic gain can be expected from selection on the sires or dams of sons pathways, which supports the results of the simulations of Sehested and Mao (38) in which genes of considerable magnitude and free of conflicting selection pressures are likely to have the frequencies of desirable alleles increased to near fixation by classical selection procedures, thus, precluding the need for genetic markers. However, within segregating families, significant benefits are possible and should be utilized because of the availability of blood group genotypes on young bulls. Considering the steady decrease in the frequency of the M_2M' allele across decades (Table 7), conflicting selection pressures are unlikely to occur on the chromosomal segment marked by the M blood group, and nothing would likely be lost by eliminating the allele from the population. Cowan et al. (11) identified the segregation of a somewhat larger quantitative effect on milk yield associated with a restriction fragment length polymorphism based on a bovine prolactin cDNA probe within a family of an elite Holstein sire. The M blood group and prolactin both map to chromosome 23 (BTA23) and are linked at a genetic distance of approximately 10 cM (8). Ashwell et al. (7) and Ron et al. (36) have also recently presented evidence confirming the presence of a QTL linked to the M blood group that impacts somatic cell count.

In the chromosomal segment marked by the L blood group, there are QTL that impact milk yield and composition traits. The magnitude of the milk yield effect is considerable; the mean of the absolute values of within-family allelic contrasts was 1122 kg (Table 12). An effect of this magnitude should prove useful for MAS and further research is warranted to fine-map the QTL, to clarify the underlying genetic model (numbers of segregating QTL, magnitudes of effects, and allele frequencies), and to design selection programs (1, 19, 20, 27, 30, 31, 33). However, the linkage disequilibrium in the population appears to be insufficient to allow the utilization of L blood group genotypes in mass selection, and phase relationships must be determined within families before MAS can be implemented. Andersson-Eklund and Rendel (2) recently reported the detection of a major gene for milk fat percentage that is linked to the amylase-1 locus. They estimated a difference between alternate (within-sire) offspring groups of 0.2% in milk fat percentage ($1 \sigma_a$; cf. corresponding magni-

tude in Table 12). Because amylase-1 and the L blood group are linked and map to BTA3 (9, 13), these effects (Tables 7 to 14) are probably detecting the same QTL.

There appears to be a QTL with a very large effect on milk fat yield on the chromosomal segment marked by the S blood group (BTA21). The magnitude of this effect was estimated to be 78.7 kg (Table 15) for the mean of the absolute values of within-family allelic contrasts, which is a major gene effect (28), and some of the segregating families exhibit remarkably large effects (Table 15). Results indicate that the effect is due to a linked QTL but that some linkage disequilibrium is present in the population. It also appears that the effect on fat yield is not accompanied by concomitant effects on milk or protein yields (Tables 10 and 11), which makes its potential usefulness unclear in payment systems that do not emphasize fat yield or percentage. However, the magnitude of the effect makes this putative QTL a candidate for research into the physiological mechanisms that underlie milk fat synthesis. The large magnitude of this effect (Table 15), which is not supported by previous research, suggests that caution is needed in the interpretation of this result.

A QTL impacting rump angle appears to be present in the chromosomal segment marked by the C blood group system on BTA18. The average effect is moderate, but, in some segregating families, large differences between alternate QTL alleles were detected (Table 15). The effect is due to a QTL linked to the blood group, but some linkage disequilibrium is present. The usefulness of this effect for MAS is not clear. In the segregating families with the greatest differences between alternate QTL alleles, selection against the undesirable allele could prove valuable. Understanding what type of gene influences the physiology of the rump angle of an animal is an aspect of this type of genetic analysis that is fascinating to contemplate (14). Physiological studies within families segregating for this QTL could perhaps be undertaken in the future.

A direct effect of the alleles of the B blood group on milk fat traits, which had been suggested by previous research, was confirmed in this study, but the magnitude of the effect is small and appears to be of limited utility for MAS schemes.

The conclusions from this study apply to blood group effects on first lactation milk traits. Although marker effects on first lactation traits are likely to be present in later lactations, the magnitude and direction of QTL effects affecting first lactation milk traits should be examined in later lactations before general

utilization. Epistatic and pleiotropic or linked effects on other important traits should also be evaluated before widespread utilization (41, 45).

Resolving Continuous Variation into Individual QTL Effects

Almost 60 yr ago, Sewall Wright commented that "separate genes cannot be classified as good or bad" [pg. 21 in (54)]. Only when the entire genotype of an animal is defined and when that animal is placed in a specific environment can a particular allele at a particular locus be judged to be favorable or unfavorable for a particular production objective in that environment (54). Thus, a considerable proportion of the quantitative effects that are assigned to genetic markers in a particular experiment may be specific to the population utilized, to the environment (micro and macro) studied, and, more seriously, to the particular combination of genotypes and environments in the experiment. Some effects are specific to certain families, and some are specific to certain families in certain environments. Even within the same experiment, statistical designs that make use of differing subsets of the available families may produce contradictory results. The underlying biological and genetic explanations for some of the inconsistencies that appeared as early as 1961 and were puzzling to Neimann-Sorensen and Robertson (29) also emerged in this study [Tables 9 to 11 and Rocha et al. (35)] and should not be overlooked in determining our expectations of MAS. Undoubtedly, the effects that are assigned to some genetic markers are relatively consistent across environments, families, and populations and have a magnitude of effect that will make them useful for MAS. However, the identification of these systems from those marker-QTL associations that are spurious or dependent on genetic architectures or environments is likely to be a demanding task.

None of these considerations should detract from current research efforts. Robertson (32) thought that theoretical developments in quantitative genetics had, in a way, come to an end, and that scientific interest in this area would eventually focus on the resolution of phenotypic variation into Mendelian components. Because much is to be gained from this approach, particularly at the interface between genetics and physiology, unrealistic expectations must not be generated within the scientific and producer communities. Both QTL mapping and utilization are intrinsically breeding and genetic problems and not simply statistical exercises (34); if the focus is on opportunities related to the strategic and timely defi-

niton of breeding objectives, on the careful evaluation and definition of environments (both production and marketing), and on the importance of alternate genetic backgrounds, this area of research will likely come to fruition.

CONCLUSIONS

Sufficient information now exists to allow the immediate utilization of M blood group phenotypes in MAS breeding decisions. Cows possessing the M₂M' allele should be viewed as candidates for culling, and young bulls with this allele should perhaps be excluded from progeny-testing schemes. Elimination of the M₂M' allele from the US Holstein population should be viewed as a desirable objective, although limited genetic gains are to be expected.

The most promising and potentially useful effect detected in this study is a QTL linked to the L blood group that impacts milk yield and composition traits. There is insufficient linkage disequilibrium to utilize this marker outside of segregating families. A major effect on milk fat yield and a moderate effect on rump angle are associated with the chromosomal segment marked by the S and C blood groups, respectively. Validation of these effects by independent studies is desirable. The integration of these marker effects into existing systems and models for genetic prediction should be considered.

Knowledge accumulated from over 40 yr of research into blood group-QTL associations with dairy traits strongly indicates the need for a conservative attitude toward the potential short-term benefits of MAS. Although short-term breeding applications should be emphasized, the integration of physiology and genetics should be the long-term objective of this area of genetic research.

Key ingredients for future breakthroughs appear to be the identification of selection objectives recalcitrant to classical quantitative genetic approaches; a production system involving considerable financial incentives; the careful definition of the target genotypes and environments; and a commitment to the implementation of a focused, disciplined, and aggressive research and breeding program.

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